2013

Phosphorus Retention and Transformation in Floodplain Forests of the Southeastern United States

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**Phosphorus Retention and Transformation in Floodplain Forests of the Southeastern
United States**

John A. Navaratnam

**Dissertation submitted to the
Eberly College of Arts and Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Doctor of Philosophy
in
Biology**

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**Keywords: Phosphorus Biogeochemistry, Wetland, Floodplain Forest, Alluvial,
Blackwater, River, Soil, DNA, Bacteria, Fungi, Isotope, ³¹P-NMR**

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ABSTRACT

Phosphorus Retention and Transformation in Floodplain Forests of the Southeastern United States

John A. Navaratnam

Phosphorus (P) commonly limits productivity in freshwater ecosystems; thus, increased P loading, either in dissolved (DP) or particulate (PP) form, can lead to eutrophication. In riverine ecosystems, floodplain forests (FFs) provide an important ecosystem service, that of improved water quality downstream, by either removing P from these incident floodwaters (retention) or by transforming P prior to export. The central goal of my dissertation research is to understand these biogeochemical mechanisms of P retention and transformation in rivers and streams and their associated FFs in the Atlantic Coastal Plain of the southeastern US (VA, NC, SC, GA).

To assess the nature of P removal during flooding events, *I conducted a detailed study of DP and PP forms (inorganic, P_i and organic, P_o) in waters from alluvial (AL) and blackwater (BW) FFs.* At each FF site, floodwaters were collected both from the river inflow point (RI), from within the floodplain (FP) near the outflow point, and analyzed P forms by ^{31}P nuclear magnetic resonance (NMR) spectroscopy. In both AL and BW samples, total DP and dissolved orthophosphate (DP_i) declined significantly from RI to FP, while dissolved pyrophosphate (complex DP_i) and total DP_o were significantly higher in FP than in RI waters. Orthophosphate monoesters comprised the majority of the DP_o pool and were significantly higher in FP floodwaters in both AL and BW systems. This suggests that in both AL and BW systems, DP_i was converted to DP_o form during flooding events, reducing its bioavailability and potential to cause the eutrophication of downstream waters.

In a second study, *I added $^{32}\text{P}_i$ dissolved in river water to soil cores collected AL and BW FFs to identify specific mechanisms of P retention and transformation during flooding.* As much as 77 % of added $^{32}\text{P}_i$ was transformed to $^{32}\text{P}_o$ within 2 h of addition in BW river waters, while in AL river waters, all added $^{32}\text{P}_i$ remained in inorganic form. However, when labeled river waters were added to soil cores, upon contact with the soil surface, additional transformation was observed resulting in an increase in the proportion of $^{32}\text{P}_o$ in headwaters, averaging 68 and 80 % of total ^{32}P recovered in AL and BW cores, respectively. Across all soil cores, average 12 and 0.5 % of the total ^{32}P added was recovered from head- and drain-waters, respectively. The remaining 87.5 % of added ^{32}P was recovered in soils, distributed within the soil fractions (73 %), microbial biomass (9 %) and roots (6 %). The proportion of $^{32}\text{P}_i$ and $^{32}\text{P}_o$ in cores averaged 69 and 31 % in AL compared to 53 and 47 % of total ^{32}P recovered in BW cores. These results suggest that floodplain soils act as a second line of defense protecting water quality, by retaining P inputs that are not initially transformed within the water column (river- and head-waters) during flooding events.

Despite our knowledge of the significant role of microorganisms in wetland soil P dynamics, little is known about the metabolic processes or taxonomic composition of the bacteria and fungi involved in the retention and transformation of P in wetlands, especially FFs. In a third study, *I compare changes in microbial community composition in AL and BW FFs.* Soil chemical properties reveal that total P (TP) concentrations were significantly higher in AL compared to BW soils, whereas total C (TC) was significantly higher in BW compared to AL FF soils. Similarly, the bacterial and fungal community composition was taxonomically different in AL compared to BW FF soils. Detrended correspondence analyses of the microbial profiles and

the subsequent fitting of environmental variables to ordination plots, revealed that TP and pH were associated with the composition of bacterial and fungal communities in AL soils, whereas, TC was strongly correlated with the microbial community profiles in BW FFs. These results indicate that differences in FF type (AL vs. BW) are associated with differences in soil TC, TP and pH, that are important predictors of microbial composition.

My findings clearly indicate that FFs are indeed valuable ecosystems that have profound effects on the biogeochemical retention and transformation of excess P loadings carried with floodwaters, playing an important role in maintaining water quality in downstream aquatic ecosystems. It is forecasted that by 2025 as much as two-thirds (an estimated 5 out of the 8 billion people) of the world population will experience severe water scarcity due to climate change impacts and poor water quality resulting from the eutrophication of potable freshwater reservoirs. North America is likely to be impacted by these trends, and as such future policy decisions may be dependent on the prioritization of riparian wetlands (particularly FFs) based on their ecosystems functions and values. Despite their importance, in the southeastern US alone, bottomland hardwood forests (including FFs) continue to be lost at a rapid rate. This destruction of FF wetlands and their associated biogeochemical function is likely to add substantially to the eutrophication problem, and will continue to do so unless measures are taken to assess the sustainability of the ecosystem services they provide, and successfully replicate them through conservation, creation, and/or restoration of these wetland types.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Mark R. Walbridge for his encouragement and support during the course of this dissertation. His editorial advice was essential to the completion of this document. I would also like to thank the members of my advisory committee; Dr. Richard B. Thomas, Dr. R. Kelman Wieder, Dr. Kevin C. Daly, Dr. William T. Peterjohn, and as well as Dr. Barbara J. Cade-Menun, for providing much-appreciated encouragement and support following Mark's departure from WVU within a year of my studies. Over the years, I have been privileged to mentor several undergraduate students with research projects, and am especially indebted to the diligent efforts made by Neha A. Kumar, Joseph D. Lynch, John L. McClannahan, and Hasan Shahid. I would also like to acknowledge the faculty, administrative staff, and graduate students of our department, both past and present. Finally, I would like to thank my family and friends for their unconditional love, patience, and unyielding support, especially during some of the more difficult times over the course of this degree.

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CHAPTER 1

General Introduction

Phosphorus, an essential macronutrient in the environment, is vital to both biological structures (phospholipid membranes) and processes (replicating polymers (DNA and RNA) and cellular metabolism (energy transfer, ATP)) (Sterner and Elser 2002). In addition to its role in the transfer and storage of energy, P is important for mechanisms involved in cell growth and reproduction in plants, bacteria, and phytoplankton. Biological productivity is contingent upon the availability of P to photosynthetic organisms, as well as microbes that form the base of the food web in both terrestrial and aquatic ecosystems (Sterner and Elser 2002, Elser et al. 2007).

Phosphorus has a special function among the substances assimilated by living things: carbon, hydrogen, oxygen, and nitrogen are the elements of biological frameworks, phosphorus is the instrument of their manufacture (Pautard 1976).

Despite its biological importance, P often limits primary production in both terrestrial (Walker and Syers 1976, Vitousek and Howarth 1991, Vitousek 2004) and freshwater aquatic ecosystems (Schindler 1978, Hecky and Kilham 1988, Carpenter et al. 1992), and despite the importance of nitrogen (N) recognized generally as a limiting nutrient in estuaries and saline systems, P has been found to limit primary productivity in some marine and brackish environments as well (Hecky 1998, Hellström 1998, Sanudo-Wilhelmy et al. 2001, Sundareshwar et al. 2003, Boesch et al. 2005, Sanudo-Wilhelmy 2006). Although P is an essential nutrient for all aquatic organisms, increased P loading to aquatic ecosystems can have negative effects. When nutrient inputs to aquatic ecosystems exceed the uptake capacity of primary producers, P loading can lead to eutrophication of freshwater and marine ecosystems. Eutrophication and its associated effects

– degraded water quality, toxic algal blooms, anoxia, and loss of aquatic biodiversity, are primarily the result of anthropogenic (or human enhanced) nutrient inputs, mainly N and P (Carpenter 2005, Dodds et al. 2009). Phosphate (or dissolved inorganic P, DIP) is a common agent of cultural eutrophication in aquatic ecosystems, and is the form most likely to increase as natural landscapes are converted to culturally dominated ones (Carpenter et al. 1998, Withers and Jarvie 2008). Eutrophication can affect entire ecosystems and due to its occurrence across a wide geographical range, is regarded as one of the largest threats to the environment today (Bennett et al. 2001, Smith 2003, Carpenter 2005).

Changes in land use have been identified as an important component of global change that can alter an ecosystem's structure, function, and interactions with the atmosphere and other terrestrial and aquatic ecosystems on the landscape (Vitousek 2004). As natural landscapes are converted to culturally dominated ones (with a significant proportion of land use in agriculture, urban/suburban development, etc.), nutrient loadings (e.g. P), particularly to aquatic ecosystems, increase (Matson et al. 1997, Basnyat et al. 1999, Carpenter 2005). In addition to land use change, fertilizer use, deforestation, increased cultivation, and urban and industrial waste disposal all have enhanced P transport from terrestrial to aquatic ecosystems by as much as a threefold increase in riverine-P flux over pre-agricultural levels (Caraco 1995, Melack 1995, Withers and Jarvie 2008). About 80 % of all nutrients in the sea come from land-based activities, including sewage and runoff from agriculture and urban areas (Ruttenberg 2005). Well over 90 % of the P delivered by rivers to the ocean is as particulate P and the remaining total dissolved P component occurs in both inorganic and organic forms, where dissolved organic P (DOP) may account for as much as 50 % of the dissolved riverine-P input (Maybeck 1982,

Ruttenberg 2005). Excess P loading has led to changes in the biodiversity and nutrient status of marine and freshwater ecosystems across a wide geographic range (Smith 2003, Elser et al. 2007). Certain plants and animals thrive with excess nutrient loading, enabling them to increase in number and geographic extent, frequently at the expense of other native or economically important species (Pretty et al. 2003, Carpenter 2005, Dodds et al. 2009). Excessive growth of filamentous algae can result in oxygen depletion that leads to fish kills and changes in fish species composition, where economically less valuable freshwater and marine fishes thrive and sustainable fisheries are severely affected (Wilson and Carpenter 1999, Carpenter 2005, Dodds et al. 2009). In addition to causing anoxia and fish kills, certain marine dinoflagellate blooms (e.g., red/brown tides in the Baltic Sea and elsewhere) produce toxins that impact biodiversity, while in freshwater systems blue/green cyanobacteria can release neuro- and hepato-toxins that kill live-stock and harm humans (Kotak et al. 1993, Hoagland et al. 2002, Carpenter 2005).

Although the vulnerability of natural waters to anthropogenic P enrichment has long been recognized, successful remediation strategies to provide a sound basis for the management of aquatic ecosystems are much needed (Fisher et al. 1995, Costanza et al. 1997). The nutrient retention and transformation capacities of riparian wetlands (including floodplain forests (FFs)) may be one mode of defense against reducing water-associated contaminants and excess nutrients (such as P), to achieve the required P load reduction in riverine systems, and mitigate the consequent eutrophication of aquatic ecosystems.

1.1 Wetland Ecosystem Services

Located in stream and river floodplains, riparian wetlands (including FFs) occupy low-lying

areas on the landscape, and as a consequence, are periodically inundated by waters from overbank flooding (Brinson 1993, Walbridge 1993, Mitsch and Gosselink 2007). As such riparian wetlands are transitional between terrestrial (upland) and open-water aquatic ecosystems, giving them the potential to regulate nutrient exchange between terrestrial and adjacent aquatic ecosystems, with beneficial effects on water quality in aquatic systems downstream (Kleiss 1996, Mitsch and Gosselink 2007). It is well known that FFs can improve water quality by removing nutrients, particularly N and P (Lowrance et al. 1984, Faulkner and Richardson 1989, Kuenzler 1989, Johnston 1991, Walbridge and Lockaby 1994, Lockaby and Walbridge 1998) from incident surface (overbank flooding – in addition to surface flows from surrounding uplands) and subsurface (ground) waters. While N is removed from soils primarily through denitrification, particularly during periods of low redox potential in wetland soils (DeLaune et al. 1996), the removal of DIP may involve several biotic and abiotic mechanisms (Walbridge 1991, Walbridge et al. 1991, Mitsch and Gosselink 2007). The P cycle is fundamentally different from the N cycle in that it has no substantial gaseous phase to facilitate the removal of P from wetlands (Reddy et al. 1999). This lack of gaseous phase means that DIP entering a FF is either stored in wetland sediment/soil or plant and microbial biomass, or remains in solution and may be carried out of the FF during flooding events (Hupp et al. 2005). Floodplain forests improve water quality by storing DIP received in floodwaters within the wetland (retention), and/or converting it in form (transformation) and then exporting it from the wetland ecosystem via hydrologic pathways (Richardson 1989, Lockaby and Walbridge 1998, Mitsch and Gosselink 2007). Thus the phosphate (DIP) carried in floodwaters that can lead to eutrophication of aquatic ecosystems is either retained within the wetland ecosystem, through sediment deposition, soil adsorption, plant uptake, or microbial immobilization (Johnston 1991,

Lockaby and Walbridge 1998, Khoshmanesh et al. 1999), or transformed to organic P form(s) and then exported downstream (Lockaby and Walbridge 1998, Noe and Hupp 2007, Dierberg and DeBusk 2008). Any DIP not processed via these pathways would pass through the wetland ecosystem and then be exported downstream as DIP. High molecular weight organic P forms are less bioavailable than DIP, because they require enzymatic hydrolysis before they can be utilized by aquatic microorganisms, and are thus less likely to cause eutrophication of downstream aquatic ecosystems (Pant et al. 2002, McDowell and Koopmans 2006). While it has been well-established that FFs can both retain and/or transform DIP (Yarbro 1983, Lockaby and Walbridge 1998, Hupp et al. 2005), little is known about the relative contributions of these P retention/transformation mechanisms or how these functions are accomplished. Understanding the detailed mechanisms of P retention and transformation in FFs is critical to assessing the sustainability of these ecosystem services, as well as replicating them through wetland creation and restoration (Kuenzler 1989). Although, FFs currently represent a substantial 83 million ha of the world's wetlands, large expanses of these wetlands have been destroyed over the past few decades, primarily for conversion (draining and clearing of forests) to agriculture and urbanization (Turner et al. 1981, Hupp et al. 2005)

1.2 Floodplain Forests of the Southeastern US

Floodplain forests are found in many landscapes worldwide (e.g., Marion et al. 1993, Schoor 1994, Cey et al. 1999, Maingi and Marsh 2001, Amoros and Bornette 2002, Capon 2005, Brunet and Astin 2008, Lu and Yin 2008) and represent 14.5 % (Aselmann and Crutzen 1989) of the global distribution of wetlands (USDA estimates an area of 83 million ha of wetlands on the Earth's surface (Mitsch and Gosselink 2007). An estimated 66, 21, and 11 % of global FFs are

found in South America, Africa, and the United States, respectively (Aselmann and Crutzen 1989). Currently, riparian forests and freshwater wetlands, which include FFs, cover 22 – 25 million ha in the conterminous US (Mitsch and Gosselink 2007). Overall, about 53 % of the wetlands in the US were lost between 1780's and 1980's, amounting to a decrease from 89 to 42 million ha over this period (Kress et al. 1996, Mitsch and Gosselink 2007). Freshwater marshes and forested wetlands were the hardest hit, with loss rates of 7 and 5 % per decade, respectively. Despite their importance, in the southern US alone, bottomland hardwood forests (including FFs) continue to be lost at a rapid rate, having declined by 6.5 million ha (77 % loss) between 1883 and 1991 (Mitsch and Gosselink 2007). This destruction of FF wetlands and their associated biogeochemical functions (e.g. nutrient retention and/or transformation capacity) has added substantially to the eutrophication problem (Carpenter 2005, Mitsch and Gosselink 2007, Dodds et al. 2009), and will continue to do so unless measures are taken to assess the sustainability of the ecosystem services they provide, and successfully replicate them through conservation, creation, and/or restoration (c.f., Erwin, 2009). In the southeastern US alone, a greater understanding of the P retention and transformation capacities of FFs would assist in making regulatory decisions as to their value in controlling eutrophication.

In the southeastern US (Virginia, North and South Carolinas, and Georgia), FF ecosystems are associated primarily with two types of river systems: 1) alluvial (AL) rivers, whose waters arise in the Piedmont or in montane physiographic provinces; and 2) blackwater (BW) rivers, whose waters arise entirely within the Coastal Plain (Wharton and Brinson 1979, Lockaby and Walbridge 1998, Hupp 2000). Blackwater rivers tend to be smaller than AL rivers, with lower gradient flows that have limited potential to entrain or erode sediments (Burke et al. 2003,

Howitt et al. 2007). Alluvial rivers have higher sediment loads and higher concentrations of inorganic ions, but lower concentrations of total organic C compared to BW rivers; pH, hardness, and specific conductance also tend to be higher in AL versus BW rivers (Wharton and Brinson 1979, Hupp et al. 2005). The floodplains of southeastern US Coastal Plain rivers are inundated periodically, often for prolonged periods. During bankfull discharge or overbank flooding, when river waters begin to overflow onto the floodplain, floodwaters impact both the biogeochemical processes and overall productivity in these wetlands (Walbridge and Lockaby 1994, Lockaby and Walbridge 1998, Mitsch and Gosselink 2007). In both AL and BW floodplains, flooding events can vary in intensity, duration, and in the number of events from year-to-year, although the probability of flooding in our FF sites in the southeastern US, where approximately 60 % of annual flooding events occur between January and April, is fairly predictable (Dress et al. *in preparation*).

The hydrology of FFs, particularly the influence of overbank flooding, is important for the maintenance of FF structure and function (Brinson 1993, Walbridge and Lockaby 1994, Hupp 2000). Flooding events can affect many biotic and abiotic patterns and processes in these ecosystems, including species composition and richness, primary productivity, organic matter accumulation, and nutrient cycling and availability (Hupp 2000, Hein et al. 2003, Hupp et al. 2005). Hydrology leads to unique vegetation composition and diversity, where depending on the frequency, duration, physical energies of floodwaters, and the hydroperiod following flooding events, flooding regimes can influence species richness (Lockaby and Walbridge 1998, Hupp 2000). The hydrology of frequently flooded wetlands acts to select water-tolerant vegetation (e.g. bald cypress), while excluding flood-intolerant species (Schlesinger 1978, Lowrance et al.

1984, Burke et al. 2003). Floodplain forests that sustain long hydroperiods (with stagnant waters), tend to have lower plant species richness than do less frequently flooded areas (Lockaby and Walbridge 1998, Hupp et al. 2005). In contrast, flowing water (sheet flow) stimulates vegetation species diversity by increasing sediment deposition and associated nutrient inputs, reducing anaerobic conditions, and creating spatial heterogeneity when the action of flowing water and sediment deposition creates a variety of ecological niches (Yarbro 1983, Lockaby and Walbridge 1998, Burke and Chambers 2003, Hupp et al. 2005).

Soil microbial communities also are constrained by both water depth and the duration and frequency of flooding events (Stoeckel and Miller-Goodman 2001, Bruland and Richardson 2005, Carreira et al. 2008). Prolonged flooding events that result in reduced soil conditions will favor microbes capable of metabolizing in anoxic conditions, but may exclude facultative anaerobes that dominate the soil during the growing season when flooding events are less-likely and soil moisture levels decline (Stoeckel and Miller-Goodman 2001, Lockaby et al. 2005). Microorganisms belonging to these two functional groups process nutrients differently when mediating P transformation and catalyzing chemical changes that control nutrient availability to plants as well as the overall productivity of wetland soils (Groffman et al. 1996, Fernandez et al. 2008). Floodplain forest hydrology also contributes to the accumulation of organic material through its influence on microbial decomposition rates, wetland primary productivity, and particulate organic matter export (Tockner et al. 1999, O'Connell et al. 2000, Neatrour et al. 2004, de Neiff et al. 2006, Turner et al. 2007). Globally, wetland soils represent an important carbon sink, and FFs are no exception, where frequent short-duration flooding events can result in the accumulation of organic matter (or peat) due to increased primary productivity and/or

decreased decomposition and organic matter export (Robertson et al. 1999, Bridgham et al. 2006). In summary, flooding events in FFs determine many factors -- soil moisture is dependent on flooding regime, while flowing waters aerate roots and transport nutrients resulting in increased productivity within the wetland, in turn affecting many biotic and abiotic factors, including species composition and richness, primary productivity, organic matter accumulation, and nutrient cycling and availability.

In addition to modifying the biological and chemical properties of FFs, overbank flooding alters the physical properties of the floodplain by entraining and eroding the sediment to create a spatially heterogeneous surface (Jones et al. 1996, Bruland and Richardson 2005, Brunet and Astin 2008, Carreira et al. 2008, Karssenberg and Bridge 2008). This topographically heterogeneous environment, in turn, has a direct influence on hydroperiod at the meso- and micro-topographic scale (Walbridge and Lockaby 1994, Stanturf and Schoenholtz 1998, Carreira et al. 2008). In general, topographic lows (swales) have comparatively long hydroperiods and fine-textured, clayey soils typical of sediments underlying low energy waters (Hupp and Morris 1990, Cowell 1993, Bruland and Richardson 2005); topographic highs (ridges), as remnants of old levees and meander scrolls, have comparatively short hydroperiods and coarse, sandy soils typical of sediments underlying high energy waters (Stoeckel and Miller-Goodman 2001). At the Ogeechee River, GA, Darke and Walbridge (2000) found that inorganic P retention and transformation processes were dominated by organic matter – metal (Al, Fe) – phosphate complexation (described under ‘P retention’ below), and were significantly greater in topographic lows (swales) within the floodplain. Total P concentrations (including microbial biomass P) were also consistently higher in swales, suggesting that swales may function as

microbial ‘hotspots’ of P immobilization (retention) and transformation (Stoeckel and Miller-Goodman 2001, Wright et al. 2001).

1.3 Biogeochemistry: Phosphorus transformation

Phosphorus in floodwaters can occur in both inorganic and organic forms, in either the dissolved/soluble (DP) or insoluble/suspended (or particulate, PP) fractions, which vary in their bioavailability in aquatic ecosystems. Dissolved and particulate P fractions are operationally defined by passing through 0.2 (colloidal P) or 0.45 μm (PP) filters, respectively. Forms of inorganic P in floodwaters can occur as particulate inorganic orthophosphate (PIP) associated with mineral sediment, and as dissolved orthophosphate which is also referred to as dissolved inorganic P (DIP) or soluble reactive P (SRP). Organic P forms include both particulate (Kutuzova et al. 2009) and dissolved organic P (DOP). Floodplain forests not only store (retain) nutrients, but also transform nutrients from biologically available forms to less biologically available forms (Nikolaidis et al. 1993, Mander et al. 1995, Lockaby and Walbridge 1998, Lockaby and Conner 1999, Maine et al. 2004, Noe and Hupp 2007).

Phosphorus pools in river waters flowing into FFs are often dominated by inorganic P forms, while those in outflow waters are often dominantly in organic P forms (e.g., (Craft and Casey 2000, Darke and Walbridge 2000, Stoeckel and Miller-Goodman 2001, Bruland and Richardson 2005, Noe and Hupp 2007). These observations suggest that the transformation of inorganic P to less bioavailable high molecular weight organic P forms is an important process during flooding events that improves water quality downstream. Both abiotic and biotic mechanisms can

regulate the relative sizes of P pools and the transformation of DIP to organic P forms in floodwaters transiting FFs. While P retention and transformation processes occur during flooding events in the water column (Evans and Johnes 2004, Benitez-Nelson et al. 2007), a substantial proportion of inorganic to organic P transformation takes place in sediments/soils, and the resultant DOP and POP forms are re-suspended by the scouring of material from the floodplain surface in subsequent sheet-flow flooding events (Reddy et al. 1999, Radke et al. 2004, Zehetner et al. 2008). One mechanism of P biogeochemical processing in FFs involves the biotic uptake or immobilization of DIP (Brinson et al. 1984, Khoshmanesh et al. 1999, Reddy et al. 1999, Hupp 2000, Stoeckel and Miller-Goodman 2001, Wright et al. 2001). Both plants and microorganisms (**Figure 1.1 a-b**) actively compete with processes of physicochemical precipitation and geochemical adsorption reactions of DIP and DOP in the water column and in the sediment/soil solution (Tate 1984, Lee et al. 1990, Reddy et al. 1999). Phosphorus uptake, storage, and release in plants is dependent on the type of vegetation, translocation of P from leaves, leaching of P from detrital tissue, and the capacity and rates of decomposition of senesced leaves, through which phosphate is subsequently released (Schlesinger 1978, Yarbrow 1983, Mitsch and Gosselink 2007). Evergreen and deciduous shrubs play a significant role in P assimilation and storage, whereas trees, which are the dominant vegetation in FFs, represent approximately 10 % of gross P uptake (Mitsch et al. 1979, Reddy and DeBusk 1987, Mitsch and Reeder 1991); annual rates of plant P uptake and storage range from 0.4 – 3.8 kg/ha in FFs of the southeastern US (cf., Lockaby and Walbridge, 1998). Although DIP is removed from solution by plants, plant uptake is predominantly a seasonal sink since net assimilation of P in wood is small when compared to leaves, with the remaining P recycled to the soil surface via litter fall decomposition (Brinson et al. 1981, Polunin 1984, Reddy et al. 1999, de Neiff et al. 2006).

Thus, over the short term, rapid turnover rates and cycling can release bioavailable P from the decomposition of plant detrital material. Microorganisms, however, play a major role in immobilizing and subsequently transforming P (upon cell lysis) in FFs (Richardson et al. 1988, Johnston 1991, Reddy et al. 1999, Stoeckel and Miller-Goodman 2001, Wright et al. 2001). Richardson et al. (1988) estimated that microbial immobilization of P ranged from 35.0 – 40.0 kg/ha/yr, which is considerably higher than plant uptake and storage of P in riparian forests in the southern US. Although microbes are generally considered decomposers that simply mineralize organic P (**Figure 1.1**), they actively incorporate (immobilize) DIP into their biomass (forming polyphosphates) from the water column and sediment/soil interface during favorable environmental conditions, but release less bioavailable POP forms upon cell lysis during unfavorable conditions (Kleeberg and Schlunbaum 1993, Khoshmanesh et al. 1999, Qualls and Richardson 2000) (**Figure 1.1 b**). Microbially produced organic P forms range from easily decomposable orthophosphate monoesters to complex molecules (i.e. phosphonates), which are of limited bioavailability and can only be utilized by a few microorganisms possessing a suit of specialized enzymes (Kononova and Nesmeyanova 2002, Dyhrman et al. 2006, Fox and Mendz 2006).

Some biologically available organic P forms may also contribute to eutrophication of downstream waters, particularly when readily available DIP forms are depleted (Chu 1946, Cotner and Wetzel 1992, Quiquampoix and Mousain 2005, Turner et al. 2005, Dyhrman et al. 2006). Low molecular weight organic P compounds (e.g. phosphate monoesters) which are labile to enzymatic hydrolysis, are preferentially utilized by heterotrophic organisms over more complex organic P forms such as orthophosphate diesters (e.g. phosphoproteins, DNA, and other

polyneucleotides) that require extensive exoenzymatic breakdown prior to uptake; as a result, the latter are less likely to contribute to eutrophication (Cembella et al. 1984a, Suzumura and Kamatani 1995, Turner et al. 2002, Turner and Newman 2005, Lovdal et al. 2007, El-Rifai et al. 2008). Phosphonates, which contain a C-P bond rather than the C-O-P bond characteristic of ester phosphates (one C monoesters and two C diesters) are more stable/resistant to biodegradation (Kolowith et al. 2001, Kononova and Nesmeyanova 2002, Dyhrman et al. 2006, Fox and Mendz 2006). Common organic P species (phospholipids, phosphonates, and nucleic acids) are products of biological activity (Selig et al. 2002, Eixler et al. 2005) that can be used to assess the importance of biological processes in P transformation in FFs.

Since organic P forms vary in their bioavailability, understanding the effectiveness of FF processes in improving water quality and the potential fate (or contribution to eutrophication) of P once it leaves the FF requires detailed information about both the identities and relative proportions of organic P forms (Cade-Menun et al. 2006). However, studies to date have determined organic P concentrations by indirect techniques; total P (TP) is generally determined by digestion and colorimetric techniques (Murphy and Riley 1962) or inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Holliday and Gartner 2007), with inorganic P [or soluble reactive phosphorus (SRP)] determined colorimetrically, and organic P as simply the difference between TP and SRP. By this method, both true organic P compounds (i.e., products of biotic synthesis such as orthophosphate monoesters and diesters) and the abiotic complexation of phosphate by organic matter via metal (Al, Fe) ligands (described under ‘P retention’ below) are identified as ‘organic P’. In addition, inorganic P forms such as pyrophosphate and polyphosphate are not molybdate-reactive (like SRP) and thus will also be designated as organic

P using colorimetric techniques. These methods may contribute to an overestimation of the amount of true organic P found in floodwaters (Darke and Walbridge 1994, McKelvie et al. 1995, Benitez-Nelson et al. 2007). More importantly, these techniques provide no information on P speciation (inorganic, organic monoesters vs. diesters, or phosphonates). Few studies have detailed the P chemistries of inflow vs. outflow floodwaters, particularly with respect to organic P (Cade-Menun et al. 2006). Therefore, in my **first experiment (Chapter 2)**, I will use a recently developed extraction technique (Cade-Menun et al. 2006) for ^{31}P nuclear magnetic resonance (NMR) spectroscopy to characterize the various forms and relative proportions of inorganic and organic P chemistries in inflow and outflow floodwaters across four alluvial and four blackwater FF systems in the southeastern US.

1.4 Biogeochemistry: Phosphorus retention

Retention of nutrients in FFs decreases the load to downstream aquatic systems, which includes lakes and the sea (Johnston 1991, Mitsch et al. 1995, Axt and Walbridge 1999, Bruland and Richardson 2004, Noe and Hupp 2007). Phosphorus retention in soils and sediments is controlled by both biological (plant uptake and microbial immobilization) and geochemical (P sorption) factors. Suspended materials (POP and PIP adsorbed to mineral sediments) are removed from the water column by sedimentation, a physical process associated with the decline in flow velocity that occurs as floodwaters encounter increased resistance from the floodplain surface (Kleiss 1996, Craft and Casey 2000, Hupp et al. 2005, Benitez-Nelson et al. 2007, Brunet and Astin 2008) (**Figure 1.1 c**). Previous research in the southeastern US also has suggested that complexation with organic matter via metal (Al, Fe) ligands may be an important mechanism of

inorganic P retention and transformation in FFs (Darke and Walbridge 1994, Axt and Walbridge 1999, Lamers et al. 2006). Given their lower rates of sediment deposition, this mechanism could be particularly important in BW FFs (Burke et al. 2003) as compared to AL systems, where sediment deposition is expected to be a major component of P retention (Darke and Walbridge 2000, Hupp et al. 2005).

Soil and sediment Al and Fe minerals range from poorly ordered (amorphous or poorly crystalline) to highly structured (crystalline) forms (Darke and Walbridge 1994, Axt and Walbridge 1999). In the acid soils typical of FFs, poorly crystalline or amorphous forms of Al and Fe (in comparison with more crystalline forms) have been shown to control the adsorption and precipitation of dissolved inorganic P because of their comparatively large surface area per unit soil volume (Lee et al. 1990, Walbridge and Struthers 1993, Darke and Walbridge 1994, Axt and Walbridge 1999, Darke and Walbridge 2000, Hogan et al. 2004, Hogan and Walbridge 2007, Fernandez et al. 2008) (**Figure 1.1 d**). In addition to simple amorphous mineral forms of Al and/or Fe, both metals form complexes with soil organic matter (OM) that can also sorb DIP (Axt and Walbridge 1999, Darke and Walbridge 2000, Hupp et al. 2005) (**Figure 1.1 d**). The formation and/or persistence of OM – metal (Al, Fe) – P complexes is strongly influenced by flooding regime (Lockaby and Walbridge 1998, Axt and Walbridge 1999, Darke and Walbridge 2000, Wright et al. 2001, Bruland and Richardson 2004, Lamers et al. 2006), suggesting that the role of complexation in P retention within FFs requires further investigation. Microbial immobilization also can result in the retention of a substantial amount of inorganic P, but this pool often turns over rapidly due to the lysis of microbial biomass during unfavorable environmental conditions (e.g., prolonged anaerobic conditions following a flooding event)

(Khoshmanesh et al. 1999, Stoeckel and Miller-Goodman 2001, Wright et al. 2001) (**Figure 1.1 b**). Although both biological and geochemical processes can be important pathways of DIP removal, little is known about how *in situ* physical and chemical factors influence the relative role that each process plays in controlling P retention and transformation in FF ecosystems. To address this, in my **second experiment (Chapter 3)**, I will assess the dominant fate(s) of inorganic P received in floodwaters by adding $^{32}\text{PO}_4^{3-}$ to soil mesocosms during simulated flooding events in the laboratory. Results from this study were used to specifically identify whether dissolved inorganic P retention is driven by mineral adsorption, organic matter – metal (Al, Fe) – P complexation, or microbial immobilization.

1.5 Microbial Community Composition and Ecosystem Function

Microbial processes are an important and yet comparatively unexplored component of P retention and transformation in FF soils. Recent attempts to describe microbial communities in floodplain soils have been limited to targeting major microbial subdivisions at the Elbe River, Germany, using group specific phospholipid fatty acid profiles (Rinklebe and Langer 2006, Rinklebe and Langer 2008), or 16S rRNA terminal restriction fragment length polymorphism in FF soils along the Danube River, Austria (Besemer et al. 2005) and Murray River, Australia (Baldwin et al. 2006). Quantification of the microbial contribution to P retention has been limited to estimating microbial biomass in floodplain soils along the Coosawatchie River, SC, USA (Stoeckel and Miller-Goodman 2001), Ogeechee River, GA, USA (Wright et al. 2001), and Falthed River, MO, USA (Ellis et al. 1998). Other studies have used enrichment techniques, such as BIOLOG ecoplates on soils collected from a FF adjacent to the Watarase River, Japan

(Kamitani et al. 2006), and culture based characterizations of specific bacterial species at Four Mile Creek, SC (Leff et al. 2003), and in the Okavango Delta, Botswana (Mubyana et al. 2003). However, detailed studies of microbial community structure using molecular techniques are comparatively rare and limited to assessing archaeal methanogenic microbes in anoxic soils in the Rhine River floodplain, The Netherlands, (Kemnitz et al. 2004), bacterioplankton communities in filterate collected from the Parana River, Brazil (Lemke et al. 2009), and biofilm communities in the Clohoge River, Ireland (Anderson, 2005). Despite the importance of spatial heterogeneity to the microbial contribution to P retention/transformation processes (Wright 1998, Stoeckel and Miller-Goodman 2001), there have been no attempts to date to understand spatial changes in the soil microbial community composition of dominant FF types in the southeastern US. Therefore, the overall focus of my research in **Chapter 4**, is to understand how floodplain type (AL vs. BW) and microtopographic relief (ridges vs. swales) control variation in microbial community composition in FFs, and how this variation, in turn, are related to the retention and transformation of P received in floodwaters.

Although Kemnitz et al. (2004) targeted archaeal populations in their soils, their investigation was limited to estimating potential methanogenic activity under flooded soils (anoxic conditions); they did not assess changes in dominant bacterial and fungal identities with variations in microsite elevation or hydroperiod within the FF. In **Chapter 4** I will develop and validate a method for assessing soil bacterial and fungal communities in FFs using a combination of two molecular approaches: (1) 16S-/18S-rDNA cloning to obtain a detailed community analysis; and (2) denaturing gradient gel electrophoresis (DGGE) to screen clone libraries and identify dominant group members. The results from this study will provide a detailed

characterization of bacterial and fungal communities in ridge (less-frequently flooded topographic highs) and swale (more-frequently flooded topographic lows) microsites in one AL (Wateree River, SC) and one BW (Black River, NC) FF. Following this initial study, I will examine additional replicates of each site type, as well as ridge vs. swale variation within each site, to compare bacterial and fungal community composition as a function of floodplain type (AL vs. BW) and microtopographic elevation (ridge vs. swale) in eight FFs (n = 4 per floodplain type). In this study, microbial community composition will be assessed in composite soils from ridge and swale microsites (3 soil samples per composite) collected at each study site.

Research Objectives

1. Characterize the various forms and relative proportions of inorganic and organic P chemistries (using ^{31}P nuclear magnetic resonance (NMR) spectroscopy) in inflow and outflow floodwaters of FFs in the southeastern US (**Chapter 2**).
2. Assess the dominant fate(s) of inorganic P received in floodwaters by adding $^{32}\text{PO}_4^{3-}$ to soil mesocosms during simulated flooding events in the laboratory (**Chapter 3**).
3. Develop and validate a method for assessing soil bacterial and fungal community structures using a combination of 16S-/18S-rDNA cloning and denaturing gradient gel electrophoresis (DGGE), to provide a detailed characterization of bacterial and fungal communities in two FFs in the southeastern US, and to compare bacterial and fungal community composition as a function of floodplain type and microtopographic elevation in eight FFs in the southeastern US (**Chapter 4**).

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Figure 1.1: Biogeochemical transformation and retention of P in the sediment and the water column in floodplain forests during flooding events. POP: particulate organic P; DOP: dissolved organic P; PIP: particulate inorganic P; DIP: dissolved inorganic P. Inorganic P inputs with floodwaters are subject to four initial fates: **a**, plant uptake; **b**, microbial immobilization and release; **c**, suspended materials (POP and PIP) removed from the water column by sedimentation; **d**, P sorption by Al and Fe minerals or P complexation with organic matter through metal (Al, Fe) ligands. Figure adapted from (Reddy et al. 1999, Worsfold et al. 2008).

CHAPTER 2

Distribution of Phosphorus Forms during Spring Flooding Events in Alluvial and Blackwater Rivers and their Adjacent Floodplain Forests in the Southeastern US

ABSTRACT

Phosphorus (P) commonly limits productivity in freshwater ecosystems; thus, increased P loading, either in dissolved (DP) or particulate (PP) form, can lead to eutrophication. In riverine ecosystems, floodplain forests (FFs) provide an important ecosystem service, that of improved water quality downstream, by either removing P from these incident floodwaters (retention) or by transforming P prior to export. To assess the nature of P removal during flooding events, I conducted a detailed study of P forms in waters from alluvial (AL) and blackwater (BW) FFs in the Atlantic Coastal Plain of the southeastern US (n=4 per river type). At each site, 12 L samples were collected both from the river inlet inflow point (RI), and from within the floodplain (FP) near the outflow point. These were filtered (0.45- μ m) and analyzed for pH, dissolved C, Al, Fe, total DP (TDP), total PP and particulate C. Dissolved and particulate samples were also characterized by ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Blackwater samples were significantly lower in pH and higher in dissolved C, Al and Fe, than AL samples. Total P averaged 86 $\mu\text{g P L}^{-1}$ (52 % PP) in BW samples, and 76 $\mu\text{g P L}^{-1}$ (70 % PP) in AL samples. Although average TDP concentrations were similar in AL and BW river waters, concentrations in AL systems were 53 % lower in FP (11 $\mu\text{g P L}^{-1}$) than in RI (23 $\mu\text{g P L}^{-1}$) waters during flooding events. Dissolved P pools were predominantly inorganic, ranging from 66 – 73 % of extracted P in RI and 58 – 59 % of extracted P in FP waters in both AL and BW systems. Across both AL and BW systems during flooding events, the sum of NMR-visible DIP forms was significantly lower in FP (58 % of extracted P) than in RI (69 % of extracted P) waters. In AL floodplains, dissolved Al concentrations were significantly lower, and dissolved organic matter content was higher, in FP than in RI waters. In combination these trends suggest organic matter – metal (Al) – phosphate complexation as a potential mechanism of P transformation in AL FFs during overbank flooding events. There were no significant differences in DP forms in BW and AL samples, but BW samples were significantly higher in particulate orthophosphate monoesters, including phytate and *scyllo*-inositol P. In both AL and BW samples, TDP and dissolved orthophosphate declined significantly from RI to FP, while dissolved pyrophosphate (3 % of extracted P in RI and 7 % of extracted P in FP waters) and total DOP (31 % of extracted P in RI and 42 % of extracted P in FP) were significantly higher in FP than in RI waters. Orthophosphate monoesters comprised the majority of the DOP pool and were significantly

higher in FP (39 % of extracted P) than in RI (29 % of extracted P) floodwaters in both AL and BW systems. This suggests that in both AL and BW systems, dissolved inorganic P was converted to organic form during flooding events, reducing its bioavailability and potential to cause the eutrophication of downstream waters.

2.1. INTRODUCTION

2.1.1 Floodplain Forests of the Southeastern US

In riverine landscapes, floodplain forests (FFs) are important locations for nutrient retention and transformation (Johnston 1991, Hupp et al. 2005, Noe and Hupp 2007). Periodic inundation by floodwaters provides an opportunity for FFs to process nutrients received from uplands outside of their immediate watersheds (Brinson 1993, Lockaby and Walbridge 1998, Mitsch and Gosselink 2007). This is particularly important in agricultural and urbanizing landscapes, where river water P concentrations tend to be elevated (Matson et al. 1997, Carpenter 2005, Hupp et al. 2005) by pollutants from both point (primarily industrial or municipal waste) and non-point (diffuse) sources (Carpenter et al. 1998, Withers and Jarvie 2008). Because P commonly limits productivity in freshwater ecosystems (Schindler 1978, Hecky and Kilham 1988, Sterner 2008), increased P loadings, either in dissolved or particulate form, often lead to eutrophication, with associated effects that include degraded water quality, toxic algal blooms, anoxia, and loss of aquatic biodiversity (Kotak et al. 1993, Carpenter 2005, Dodds et al. 2009). By removing P from these incident floodwaters (hereafter referred to as retention) (Yarbro 1983, Brinson 1993, Walbridge and Struthers 1993, Darke and Walbridge 2000, Bruland and Richardson 2005, Noe and Hupp 2005) or transforming P prior to export (Richardson 1989, Lockaby and Walbridge 1998, Hupp et al. 2005, Mitsch and Gosselink 2007), FFs provide an important ecosystem service, that of improved water quality downstream (Walbridge 1993, Brinson and Rheinhardt 1996, Lockaby and Walbridge 1998, Hupp et al. 2005).

A number of studies have examined rates of P retention and transformation in FFs (Mitsch et al. 1979, Brinson et al. 1984, Craft and Casey 2000, Stoeckel and Miller-Goodman 2001, Bruland

and Richardson 2005, Noe and Hupp 2007), and yet none have detailed the various forms and concentrations of P, particularly organic P, in inflow versus outflow floodwaters during overbank flooding. Studies using state-of-the-art measurements to quantify changes in inorganic and organic P forms during sheet-flow flooding events should be of vital interest in understanding the functions of FF ecosystems.

Most previous research on P retention and transformation processes in forested wetlands has focused on floodplains of alluvial (AL) river systems in the southeastern US (Yarbro 1983, Brinson 1993, Walbridge and Struthers 1993, Darke and Walbridge 2000, Stoeckel and Miller-Goodman 2001, Noe and Hupp 2005). Alluvial rivers, whose waters arise in the Piedmont or montane physiographic provinces, generally have higher sediment loads and higher concentrations of inorganic ions (including P) than blackwater (BW) rivers (Wharton and Brinson 1979, Hupp et al. 2005), the second most common type of river system in the southeastern US. Blackwater rivers are relatively unstudied with respect to P processing during flooding events. Overall, BW systems tend to be smaller in drainage area than AL systems, with lower gradient flows and limited potential to entrain or erode sediments. Thus they are likely to receive relatively low concentrations of sediment-associated PP inputs during flooding events (Burke et al. 2003, Howitt et al. 2007). In addition, BW river waters have high concentrations of dissolved organic matter released from terrestrial vegetation and/or soils, and thus they also typically have higher concentrations of total organic C, but lower pH, hardness, and specific conductance, than AL river waters (Wharton and Brinson 1979, Hupp et al. 2005).

2.1.2 Phosphorus Forms and Mechanisms of Phosphorus Retention and Transformation

Phosphorus in floodwaters can occur in both inorganic and organic forms that vary in their bioavailability in aquatic ecosystems. These forms can be found in both the dissolved/soluble (DP) and the suspended/insoluble (particulate, PP) fractions (**Figure 2.1**). Phosphorus forms are usually defined operationally, by filtration through 0.2 (colloidal P) or 0.45 μm (PP) filters. Inorganic P forms in floodwaters include particulate inorganic phosphate (PIP), associated with mineral sediments, and dissolved inorganic P (DIP), which includes dissolved orthophosphate (primarily HPO_4^{2-} or H_2PO_4^- at the pH of floodwaters; **Figure 2.2 a**), also referred to as soluble reactive P (SRP) or molybdate reactive P (MRP), as well as dissolved complex inorganic P forms such as pyrophosphate and polyphosphate (**Figure 2.2 b, c**). Organic P forms include both particulate (Kutuzova et al. 2009) and dissolved (DOP) organic P (**Figure 2.1**). A number of studies have found that P pools in river waters flowing on to a floodplain often are dominated by inorganic P forms, while P pools in outflow floodwaters are dominated by organic P forms (Elder 1985, Craft and Casey 2000, Darke and Walbridge 2000, Stoeckel and Miller-Goodman 2001, Bruland and Richardson 2004, Van Der Lee et al. 2004, Bruland and Richardson 2005). Therefore, FFs are frequently thought of both as net sinks for inorganic P and net sources of organic P (Richardson et al. 1988, Brinson 1993, Lockaby and Walbridge 1998, Darke and Walbridge 2000, Bruland and Richardson 2004, Hupp et al. 2005).

In both the water column and at the sediment/soil interface, retention and transformation of DIP can occur by both biological (plant uptake and microbial immobilization) and geochemical (P sorption) processes. Suspended materials (both inorganic and organic P associated with mineral sediments, POP and PIP, and colloidal P) may be removed from the water column by

sedimentation, a physical process associated with the decline in flow velocity that occurs as floodwaters encounter increased resistance from the floodplain surface (Kleiss 1996, Craft and Casey 2000, Hupp et al. 2005, Benitez-Nelson et al. 2007, Brunet and Astin 2008). Sedimentation of PP is accompanied by the sorption and precipitation of DIP bound to amorphous (or poorly crystalline) aluminum (Al) and iron (Fe) oxides (Lee et al. 1990, Walbridge and Struthers 1993, Darke and Walbridge 1994, Axt and Walbridge 1999, Darke and Walbridge 2000, Hogan et al. 2004, Hogan and Walbridge 2007, Fernandez et al. 2008). In addition to simple amorphous mineral forms of Al and/or Fe, both metals form complexes with soil organic matter (OM) that can also sorb DIP, forming OM – metal (Al, Fe) – P compounds (Axt and Walbridge 1999, Darke and Walbridge 2000, Hupp et al. 2005).

Dissolved inorganic P entering FFs via overbank flooding also can be transformed (by uptake and subsequent conversions to organic form) through biosynthetic pathways associated with plants and/or microorganisms, and then exported downstream as DOP and/or POP. Microbial immobilization may also retain a substantial amount of DIP, but this pool can turn over rapidly due to the lysis of microbial biomass during unfavorable environmental conditions, such as prolonged anaerobic conditions following a flooding event (Khoshmanesh et al. 1999, Stoeckel and Miller-Goodman 2001, Wright et al. 2001). Although microorganisms generally mineralize organic P (releasing DIP), they also regulate P flux across the sediment-water interface (Kleeberg and Schlunbaum 1993, Qualls and Richardson 2000) and contribute to P burial through production of POP forms, some of which are refractory (Gachter and Meyer 1993). Although both biological and geochemical processes can be important pathways of DIP removal, little is known about the relative contribution that each process plays in transforming P during

flooding events, or the relative proportion of organic P forms that result from these transformations. Using state-of-the-art techniques to examine changes in inorganic and organic P forms in floodwaters transiting a forested floodplain could provide valuable information about the relative importance of these various processes.

2.1.3 Techniques for Determining Phosphorus in Water

Routinely, organic P in aqueous samples is quantified indirectly: Total P (TP) is determined colorimetrically (Murphy and Riley 1962) following digestion, or by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Holliday and Gartner 2007), and then organic P calculated as the difference between total and inorganic P (dissolved/molybdate reactive P (D-/MRP), measured colorimetrically before digestion) (**Figure 2.1 a**). Complex inorganic P forms such as pyrophosphate and polyphosphate (**Figure 2.2 b-c**) are not molybdate-reactive, and are thus also identified as organic P when inorganic P is determined colorimetrically. Additionally, both “true” organic P compounds (e.g., orthophosphate monoesters and diesters, and phosphonates) and OM – metal (Fe/Al) – P complexes would be identified as ‘organic P’. Thus, traditional methods are not able to distinguish between important inorganic and organic P forms that may be present in a water sample (**Figure 2.1 a**). In fact, these methods likely overestimate the amount of “true” organic P present in floodwaters or other water samples (Darke and Walbridge 1994, McKelvie et al. 1995, Benitez-Nelson et al. 2007), while providing little information on P speciation, particularly in terms of organic P forms.

Because in nature, all P is found as the stable isotope ^{31}P and is therefore NMR-visible, solution ^{31}P -nuclear magnetic resonance (NMR) spectroscopy can identify species of both organic and inorganic P in floodwaters (Cade-Menun et al. 2006) (**Figure 2.1 b**), allowing the determination of a range of P forms with a single extraction and analysis protocol. The first use of ^{31}P -NMR spectroscopy in determining organic P forms in environmental samples was by Newman and Tate (1980). Since then, solution ^{31}P NMR has become an increasingly powerful tool for the characterization of organic P in agricultural and forest soils, marine and lake sediments, manure, compost, and sludge (c.f. Cade-Menun 2005b, a, Worsfold et al. 2008). Although solution ^{31}P NMR has been used extensively for identifying P forms in extracts of soils and sediments, there have been relatively fewer applications of this technique in marine (Clark et al. 1999, Kolowith et al. 2001) or freshwater (Nanny and Minear 1994, 1997, Selig et al. 2002) samples, particularly in FFs and associated river waters (Cade-Menun et al. 2006). An improvement in the use of ^{31}P NMR spectroscopy to characterize dissolved forms of P in freshwaters (Cade-Menun et al. 2006) facilitates these types of analyses.

Since organic P forms vary in their bioavailability (Pant et al. 2002, Turner et al. 2003a, Cade-Menun et al. 2006, McDowell and Koopmans 2006, Worsfold et al. 2008), understanding the effectiveness of FF processes in improving water quality (and the potential fate of P once it leaves the floodplain) requires detailed information about both the identities and concentrations of organic P species (Cade-Menun et al. 2006). While simple DIP forms like orthophosphate are readily available for biological uptake, increasing the risk of eutrophication, some biologically available organic P forms can also cause eutrophication of downstream waters (Chu 1946, Cotner and Wetzel 1992, Quiquampoix and Mousain 2005, Turner et al. 2005b, Turner and

Newman 2005, Dyhrman et al. 2006). Most organic P forms identified in freshwater ecosystems are ester phosphates (mono- or diesters) that vary in their bioavailability to aquatic organisms (Cembella et al. 1984a, Suzumura and Kamatani 1995, Lovdal et al. 2007). When orthophosphate concentrations are low, monoesters (**Figure 2.2 d**) are preferentially utilized by heterotrophic organisms over more complex forms (e.g., diesters -- phosphoproteins, DNA, and other polynucleotides) (**Figure 2.2 e**), which require extensive exoenzymatic breakdown prior to uptake. As a result, these forms are less likely to contribute to eutrophication of aquatic ecosystems (Turner and Newman 2005, El-Rifai et al. 2008). Phosphonates, which contain a C-P bond rather than the C-O-P bond characteristic of ester phosphates, are more stable and therefore resistant to biodegradation (Kolowitz et al. 2001, Kononova and Nesmeyanova 2002, Dyhrman et al. 2006, Fox and Mendz 2006) (**Figure 2.2 f**). Because organic P species are the products of biological activity (Selig et al. 2002, Eixler et al. 2005), their presence would suggest the importance of biological processes in FF P transformations. Plant material in agricultural systems can contain significant amounts of orthophosphate diesters (52 % in nucleic acids and 23 % in phospholipids) and the remainder (25 %) as orthophosphate monoesters (Magid et al. 1996). Microbial (bacterial and fungal) biomass has been shown to contain large but variable amounts of polyphosphates, including pyrophosphate (the smallest form of polyphosphate) (Kornberg et al. 1999, Makarov et al. 2005). Increases in these compounds in outflow floodwaters would suggest the importance of plant and/or microbial processes as mechanisms of P transformation.

2.1.4 Objectives and Hypotheses

I examined changes in the forms of P in floodwaters during overbank flooding events in eight AL and BW FFS (n = 4 per river type) in the southeastern US Coastal Plain, to determine if BW and AL floodplains retain and/or transform P during flooding events (Spring 2005), and if river type affects these processes. Surface waters were sampled both at the inflow or river inlet (RI), and within the FF (FP), prior to the point of export to downstream aquatic ecosystems (i.e, outflow waters); concentrations of inorganic and organic P forms in both dissolved and particulate fractions were quantified by ^{31}P NMR spectroscopy (Cade-Menun et al. 2006). I hypothesized that:

- 1. In both AL and BW systems, floodwater TP would be significantly lower in outflow (FP) vs. inflow (RI) floodwaters, suggesting that DP and/or PP received in floodwaters is retained via biotic and abiotic processes within the FF prior to export downstream.**

I tested this hypothesis against two alternative hypotheses – **that TP entering the floodplain via overbank flooding:**

- 1a. would be significantly higher in FP than in RI waters, due to the mobilization and re-suspension of DP and PP by the scouring of material and associated P from the floodplain surface.**
- 1b. would not differ significantly between RI and FP waters.**
- 2. In both AL and BW systems, DIP in FP waters will be significantly lower, and DOP significantly higher, than in RI waters, suggesting that P transformation occurs via biotic synthesis (conversion of Pi to Po).**

This hypothesis will be tested against an alternative hypothesis:

- 2a. Where DIP forms would be significantly higher and DOP forms would be significantly lower in FP vs. RI floodwaters, due to microbial mineralization of DOP and mobilization of DIP from surface soil/sediment organic matter or dissolved organic substances containing P.**
- 2b. DIP and DOP forms would not differ significantly between RI and FP waters.**
- 3. *Because of the characteristically high sediment loads, the majority of inorganic and organic P in AL river waters would likely be present in particulate form, with sedimentation likely to be the primary mechanism of particulate phosphorus removal during flooding events.***

This hypothesis will be tested against two alternative hypotheses – **that PP concentration in floodwaters:**

- 3a. would be significantly higher in FP than in RI waters, suggesting sedimentation as the dominant mechanism of P retention.**
- 3b. would not differ significantly between RI and FP waters during high discharge flooding events where flow velocities are too high for PP sedimentation to occur.**

2.2 METHODS

2.2.1 Site Description, Sample Collection and Preparation

Water samples were collected from AL and BW FFs ($n = 4$ per river type) in the Atlantic Coastal Plain of the southeastern US (**Table 2.1**) during March and April, 2005, during peak flood stage as indicated by USGS gauge data (<http://water.usgs.gov/waterwatch/>). The AL rivers sampled in this study were significantly larger in drainage area and mean daily stream flow than BW rivers. At each site, grab samples (one 12 L sample per location) were collected from the river inflow point (RI) or, near the upstream end of the wetland, and from within the floodplain (FP), near the downstream (outflow) end of the wetland, just prior to the point where floodwaters reentered the river channel. Within 48 hr of collection, 11 L of each sample were filtered through 0.45 μm membrane filters (500 mL per filter, Cat# 141118, Whatman Inc., Clifton, NJ, USA) by vacuum filtration, to separate dissolved from particulate P. Ten L of each filtrate were frozen in ice cube trays and subsequently lyophilized (FreeZone 12 L console, Labconco, Kansas City, MO); 1 L was retained for chemical analysis; filters were refrigerated for later processing. A 500-mL subsample of the remaining unfiltered water was sequentially filtered through 0.45 μm , followed by 0.20 μm (Pall polypropylene) membrane filters, to separate particulate and colloidal, P respectively. The remaining 500 mL was used for other chemical analyses.

2.2.2 Chemical Analyses

Water samples were analyzed for pH, conductivity, dissolved and particulate organic C, total P (TP), MRP, aluminum (Al), calcium (Ca), and iron (Fe). Molybdate reactive P concentrations were measured colorimetrically on a Bran & Luebbe autoanalyzer (Norderstedt,

Germany) by the molybdate blue color reaction (Murphy and Riley 1962). Total P, Al, Ca, and Fe concentrations in filtrate solutions were measured on an ICP-AES (Thermo-Jarell Ash; IRIS Advantage). Particulate P (PP) retained on the 0.45 μm filters was quantified colorimetrically, after filters were oven-dried and digested (Parkinson and Allen 1975). Dissolved organic C (DOC) concentrations were determined on a Shimadzu TOC analyzer (Shimadzu, Columbia, MD). Particulate organic C (POC) concentrations on the 0.45 μm filters were measured on a Carlo Erba NA1500 Series II elemental analyzer.

2.2.3 NMR Extraction and Analysis

Lyophilized water samples were extracted in 25 mL of a 1:1 solution of 0.5 M NaOH and 0.1 M Na₂EDTA overnight, and then centrifuged for 20 min at 3700 rpm (Cade-Menun and Preston 1996, Cade-Menun et al. 2002, Turner et al. 2005a). For each water sample, fifteen of a total of twenty-two filters (not dried) were similarly extracted (5 filters per 25 mL of extractant) and then combined to produce 75 mL of extract. A 1-mL aliquot of the combined extract (diluted to 10 mL) of each solution was analyzed by ICP-AES to determine total extracted P and Ca; and the remaining 74 mL were lyophilized. Lyophilized samples were dissolved in a solution of 1.2 mL (particulate samples) or 0.8 mL (dissolved P samples) deionized water, 0.8 mL deuterium oxide, and 0.4 mL 10 M NaOH, then allowed to stand at room temperature for 20 min with periodic vortex mixing, and then centrifuged at 3700 rpm for 20 min. Spectra were acquired within 12 h of sample preparation at 242.75 MHz on a Varian^{UNITY} INOVA 600 MHz spectrometer equipped with a 10-mm broadband probe. Parameters were: 20 °C; 90° pulse; 0.68 s acquisition time; 4.32 s relaxation delay; and 12 Hz spinning; collecting 5800 scans for PP and 8500 scans for DP samples (Cade-Menun et al. 2006). Forms of P were identified by their

chemical shifts (ppm) relative to an external standard (orthophosphoric acid); the orthophosphate peak for each sample was standardized to 6 ppm (**Figure 2.3 and 2.4**). Peaks were processed using NUTS software (Acorn NMR, Livermore, CA, 2000 edition) with 7 Hz line-broadening for coarse identification and 2 Hz line-broadening for fine identification; peak identification was based on the literature (Cade-Menun 2005b, Turner et al. 2005b) and spiking of related samples (Cade-Menun et al. 2006).

2.2.4 Statistical Analysis

Chemical composition, drainage area, and mean daily stream flow (<http://water.usgs.gov/waterwatch/>) of AL and BW rivers were compared using a two-sample t-test analysis ($p = 0.05$). Differences in the chemistries of RI and FP water samples were analyzed by two-way ANOVA ($p = 0.05$), with main effects of floodplain type (AL vs. BW), location on each FF (RI vs. FP), the interaction term, and floodplain type nested within site. Data were log transformed where necessary to meet assumptions of normality. All statistical analyses were performed using SYSTAT version 12 (SSI, San Jose, CA). Accumulation or retention rates of P forms were calculated using the following equation:

$$\% \text{ retention of riverine P load} = [(\% \text{ P in RI} - \% \text{ P in FP}) / \% \text{ P in RI}] \times 100$$

2.3 RESULTS

2.3.1 Alluvial vs. Blackwater Rivers

Results for the chemical characteristics of individual AL and BW rivers are listed in **Table 2.2 a**. River water pH ranged from 5.3 to 7.0, and when averaged across river types was significantly higher in AL (6.4 ± 0.6) than in BW (5.6 ± 0.2) rivers (floodplain type: $F = 24.272$, $p = 0.003$, **Appendix Table 2.1 a**). As expected, dissolved organic C concentrations were significantly higher in BW than in AL systems ($F = 9.856$, $p = 0.020$, **Appendix Table 2.1 a**). Across sites and samples, Al concentrations were significantly higher than Fe concentrations, with the exception of the Pee Dee River, SC, and the FP sample from the Ocmulgee River, GA. Aluminum concentrations were also significantly higher in BW than in AL systems (floodplain type: $F = 12.787$, $p = 0.012$), and lowest in FP samples in the AL system (RI/FP location: $F = 13.602$, $p = 0.010$ and interaction between the two factors: $F = 13.735$, $p = 0.010$) (**Appendix Table 2.1 a, Figure 2.6 b**). Dissolved Fe varied widely (10.8 to $490.4 \mu\text{g Fe L}^{-1}$) and when averaged was significantly higher, by as much as ten fold, in BW ($305.7 \pm 97.2 \mu\text{g Fe L}^{-1}$) compared to AL ($31.1 \pm 11.9 \mu\text{g Fe L}^{-1}$) river waters (floodplain type: $F = 11.676$, $p = 0.014$, **Appendix Table 2.1 a, Figure 2.6 e**). Total P in AL river waters, calculated as the sum of the DP and PP fractions, was $75.7 (\pm 14.4) \mu\text{g P L}^{-1}$, of which 70 % was in particulate form. Total P in BW river waters was $86.2 (\pm 44.8) \mu\text{g P L}^{-1}$, of which only 52 % was found in particulate form (**Table 2.2 a**).

Typical spectra from NMR experiments are shown in **Figures 2.3 – 2.4**. An expanded view of a P spectrum is depicted in **Figure 2.5**, showing more detail in the orthophosphate, orthophosphate

monoester and diester, and pyrophosphate regions and is representative of the P forms observed in flood waters in this spectra region in all samples. A majority of the peaks representing specific P compounds and compound groups was identified in a previous study (Cade-Menun et al. 2006) by spiking river water samples (Pee Dee River, SC) with analogous standard inorganic and organic P compounds. All spectra show peaks for the P compound groups (from left to right): inorganic orthophosphate (6.5 to 5.5 ppm); organic orthophosphate monoesters (5.5 to 3.0 ppm); orthophosphate diesters (ranged from 3.0 to -0.1 ppm); and inorganic pyrophosphate (-4.0 to -4.3 ppm) (**Figure 2.2 a, b, d, e; and 2.5**). In the orthophosphate monoester chemical shift range, phytate (salts of *myo*-inositol hexakisphosphate, **Figure 2.2 d**) has four peaks, which were found in these samples at 5.2, 4.3, 3.9, and 3.7 ppm (**Figure 2.5**). Identification of phytate in these samples was based on the presence of the P2 peak at 5.2, and its area was multiplied by six to determine the total phytate peak area, as per Turner (2004).

Inositol phosphates, including *scyllo*-inositol phosphates (3.3 ppm, **Figure 2.5**), are highly-stable P-rich molecules having a high anionic charge. These compounds adsorb strongly to clays, mineral surfaces, and organic matter in soil and sediment, thus resisting hydrolysis and biodegradation (Turner and Richardson 2004). Additionally, signals in the monoester region include degradation products of orthophosphate diesters such as mononucleotides resulting from the hydrolysis of RNA, or glycerophosphates (GP at 4.7 ppm, **Figure 2.5**), which are degradation product of phospholipids (Doolette et al. 2009). Orthophosphates diesters were detected in all samples (**Table 2.2 b, c**) and divided into DNA at 0.1 ppm and “other” diesters (3.0 to 0.5 ppm), which include RNA and intact phospholipids (**Figure 2.2 e; and 2.5**). Both polyphosphates (**Figure 2.2 c**) and phosphonates (**Figure 2.2 f**) were detected in some samples,

but their relative contribution to total P in floodwaters ranged from undetectable to 1.3 % of extracted P (**Table 2.2 b, c**).

As measured by ^{31}P -NMR analysis, in both the dissolved and particulate fractions, inorganic P (orthophosphate + pyrophosphate + polyphosphate) was the largest constituent of the total P pool; and inorganic P concentrations were similar in AL and BW rivers. Orthophosphate in both DIP and PIP ranged from 52.1 to 77.5 % of total P. MRP content in AL ($35.1 \pm 8.9 \mu\text{g L}^{-1}$) and BW ($22.1 \pm 5.9 \mu\text{g L}^{-1}$) unfiltered river waters were consistent with concentrations of orthophosphate detected by NMR analysis. Although the majority of P in both river types was in inorganic form, a variety of organic P forms (phosphonates, orthophosphate monoesters, and orthophosphate diesters) was also present (**Table 2.2 b, c**). In both dissolved and particulate P fractions, the sums of both orthophosphate monoesters and orthophosphate diesters were consistently higher in BW compared to AL rivers, although differences were only statistically significant for the particulate orthophosphate monoesters: *scyllo*-inositol phosphate (*scyllo*-IP) and glycerophosphate (Schoch et al.) (**Appendix Table 2.1 c**).

2.3.2 River Inlet versus Floodplain Waters:

2.3.2.1 Recovery of total dissolved and particulate P in floodwaters

In total, lyophilization of 10 L of RI filtrate produced 361.7 mg (AL) and 437.1 mg (BW) of dried powder (particulate fraction); and lyophilization of FP filtrate produced 411.9 mg (AL) and 472.7 mg (BW) of dried powder. Total DP recovered following NaOH-EDTA extraction

averaged 41 % across all water samples, and was comparable to the average recovery of Ca (49 %) in these waters. Dry weights of particulates from 10 L of water were 177.7 mg (RI) and 168.5 mg (FP) in AL samples, and 101.3 mg (RI) and 105.8 mg (FP) in BW systems. After extraction with NaOH-EDTA, the average recovery of PP was 67 % across all water samples collected.

2.3.2.2 Dissolved P Forms

Total P (measured by ICP-AES) concentrations (calculated as the sum of the DP and PP fraction, **Table 2.2 a**) were similar in both floodplain types, averaging $75.7 \pm 14.4 \mu\text{g L}^{-1}$ in RI and $84.6 \pm 21.2 \mu\text{g L}^{-1}$ in FP for AL systems, and $86.2 \pm 44.8 \mu\text{g L}^{-1}$ and $85.2 \pm 36.5 \mu\text{g L}^{-1}$ for RI and FP, respectively, for waters collected at the BW systems. Although TDP did not differ between RI and FP locations in the BW systems, there was a significant decrease in TDP in FP ($10.8 \pm 2.7 \mu\text{g L}^{-1}$) compared to RI ($22.8 \pm 5.4 \mu\text{g L}^{-1}$) waters (**Table 2.2 a**) in the AL systems (two-way ANOVA, floodplain type $\times$ RI/FP location interaction: $F = 15.912$, $p = 0.007$, **Appendix Table 2.1 a** and **Figure 2.6 a**). The proportion of total P in colloidal ($< 0.20 \mu\text{m}$) form showed little variation between floodplain types, averaging $11.7 \pm 6.0 \mu\text{g L}^{-1}$ in RI and $8.1 \pm 1.9 \mu\text{g L}^{-1}$ in FP for AL systems, and $9.0 \pm 5.1 \mu\text{g L}^{-1}$ in RI and $15.2 \pm 6.6 \mu\text{g L}^{-1}$ in FP waters collected at BW systems. Colloidal P did not differ by floodplain type ($F = 0.105$, $p = 0.756$), RI/FP location ($F = 0.207$, $p = 0.665$), or an interaction between the two factors ($F = 2.990$, $p = 0.134$).

In both AL and BW systems, the majority of DP was inorganic (**Table 2.2 b**), and the proportion of DIP forms (measured by NMR) also significantly decreased from 69.3 ± 6.1 % in RI to 58.3 ± 8.9 % in FP waters (location effect: $F = 6.449$, $p = 0.044$, **Appendix Table 2.1 b** and **Figure 2.7 a**), largely due to significantly higher dissolved orthophosphate concentrations in RI (65.6 ± 5.9 %) than in FP (51.5 ± 4.8 %) waters (RI/FP location effect: $F = 10.145$, $p = 0.019$, **Appendix Table 2.1 b** and **Figure 2.7 b**). Dissolved pyrophosphate was present in most samples at levels as high as 13.6 % of extracted P (**Table 2.2 b**), but showed the opposite trend to orthophosphate, and was significantly higher in FP (6.7 ± 1.4 %) than in RI (3.4 ± 1.1 %) waters (RI/FP location effect: $F = 9.996$, $p = 0.020$, **Appendix Table 2.1 b** and **Figure 2.7 c**) in both AL and BW river systems.

Although the majority of DP was orthophosphate, which decreased significantly in FP vs. RI waters, the sum of DOP forms significantly increased in FP (41.7 ± 5.9 %) compared to RI (30.6 ± 6.1) waters (RI/FP location effect: $F = 6.449$, $p = 0.044$, **Appendix Table 2.1 b**, **Figure 2.7 d**) in both AL and BW systems. Orthophosphate monoesters were the largest constituents of the DOP fraction, averaging 25.7 to 39.7 % of extracted P (**Table 2.2 b**). Both dissolved orthophosphate monoesters and diesters were higher in FP waters in both AL and BW systems, though only the differences in monoesters (39.0 ± 5.8 and 28.8 ± 3.1 % in FP and RI waters, respectively) were statistically significant (RI/FP location effect: $F = 5.831$, $p = 0.048$, **Appendix Table 2.1 b**, **Figure 2.7 e**) in both FF types. Although most forms of dissolved orthophosphate monoesters, phytate, scyllo-IP, glycerophosphate, and other monoesters) generally increased (but were not statistically significant) in FP waters, phytate concentrations were significantly higher in FP (6.2 ± 2.0 %) than in RI (2.6 ± 1.3 %) waters (RI/FP location

effect: $F = 18.127$, $p = 0.005$, **Appendix Table 2.1 b**, **Figure 2.7 e**) in both AL and BW floodplain forests. Phosphonates and inorganic polyphosphates were detected in some samples, but their relative contributions to total P ranged from 0.0 to 1.3 % (**Table 2.2 b**).

2.3.2.3 Particulate P Forms

Total PP concentrations did not differ significantly between RI and FP waters in AL or BW systems (**Appendix Table 2.1 a**). Similarly, the sum of NMR visible PIP and POP pools were similar between sampling locations within FFs, and between AL and BW floodplain systems (**Appendix Table 2.1 c**). However, concentrations of particulate orthophosphate monoesters were significantly higher in BW than in AL systems (floodplain effect: $F = 6.847$, $p = 0.040$, **Appendix Table 2.1 c**, **Figure 2.8 a**). Individual forms of particulate orthophosphate monoesters were generally higher in BW than in AL systems, although only differences in particulate *scyllo*-IP (RI/FP location effect: $F = 13.040$, $p = 0.011$, **Appendix Table 2.1 c**, **Figure 2.8 b**) and phytate (floodplain type $\times$ RI/FP location interaction: floodplain $F = 6.000$, $p = 0.049$, **Appendix Table 2.1 c**, **Figure 2.6 d**) were statistically significant.

2.4. DISCUSSION

While a number of studies have examined rates of P retention and transformation in FF soils (e.g., Mitsch et al. 1979, Brinson et al. 1984, Craft and Casey 2000, Stoeckel and Miller-Goodman 2001, Bruland and Richardson 2005, Noe and Hupp 2007), and to a lesser degree in floodwaters (Wright et al. 1996, Valett et al. 2005, Krah et al. 2006, Noe and Hupp 2007, Loeb et al. 2008, Tian et al. 2008, Weilhoefer et al. 2008), little is known about the relative contribution of biological and geochemical retention and/or transformation mechanisms in improving downstream water quality, or how these processes vary across the major FF types associated with AL and BW rivers in the southeastern US. My estimates of TP concentrations in river water, during spring (March – April, 2005) flooding, averaging $75.7 \mu\text{g P L}^{-1}$ in AL and $86.2 \mu\text{g P L}^{-1}$ in BW rivers, fall within the range of values recorded for these rivers (USGS-Water Resources Archive, **Table 2.1**), as well as those observed in other AL and BW rivers in the southeastern US Coastal Plain (c.f., Lockaby and Walbridge 1998, Hupp et al. 2005, Noe and Hupp 2009). Total P concentrations did not differ significantly between river types. The majority of the TP in both river types studied here was present in particulate form, and the differences in TPP concentrations (measured by chemical analysis) between AL and BW rivers were statistically indistinguishable (**Table 2.2 a**). The range of TDP concentrations in both AL ($16.3 - 34.0 \mu\text{g P L}^{-1}$) and BW ($8.0 - 97.7 \mu\text{g P L}^{-1}$) rivers is consistent with DP measured in other rivers in the southeastern US Coastal Plain (c.f., Lockaby and Walbridge 1998, Hupp et al. 2005). Average TDP concentrations were also statistically indistinguishable in AL and BW rivers. Despite the inherent differences between AL and BW rivers, TP, TDP, and TPP concentrations were similar, and as a result, rates of inorganic P retention will be dependent on how physical, geochemical, and biological processes differ in their associated FFs.

Within AL floodplains, TDP concentrations significantly decreased as RI waters entered the FP during flooding events, suggesting that 53 % (ranging from 39 – 68 %) of the riverine TDP load was retained (**Table 2.2 a, Figure 2.6**). These estimates are comparable to values reported by Noe and Hupp (2009) across seven floodplains in the Chesapeake Bay watershed, USA (59 % retention rate), and in floodplains of the River Adour, southwestern France (60 – 90 % retention) (Brunet and Astin 2000). No statistically significant changes in TDP between RI and FP were detected in BW samples. Noe and Hupp (2009) suggested that larger floodplain areas and longer flood durations increase retention rates of river P inputs. Thus, the efficiency of DIP removal from the water column may in part depend on increased biogeochemical reactivity due to a larger physical contact area between floodwaters and the FF surface. As is typical in the southeastern US Coastal Plain, AL systems in this study had significantly larger drainage areas, up to 8.7 fold larger than BW floodplains, providing the first line of evidence for greater TDP retention capacity during high-discharge flooding events in AL systems (**Table 2.1**) (Lockaby and Walbridge 1998, Hupp et al. 2005).

2.4.1 P Transformation in Floodplain Forests:

2.4.1.1 ^{31}P NMR Detected Inorganic P Forms in Floodwaters

As in most environmental samples (Filippelli 2008, Liu et al. 2008), majority of the extracted DP pool in floodwaters was inorganic P, ranging from 66.0 – 72.7 % of extracted P in RI and 57.8 to 58.8 % of extracted P in FP waters at AL and BW FFs (**Table 2.2 b**). In addition, the sum of NMR-visible DIP forms decreased significantly in FP as compared to RI waters during flooding

events, suggesting transformation rates of 21 and 11 % of river DIP loads in AL and BW systems, respectively (**Figure 2.7 a**). Brunet and Astin (2000) reported an average 7.4 % of DIP (or MRP) retention within River Adour floodplains during spring flooding. Reactive orthophosphate (MRP, measured colorimetrically) behaved similarly and decreased in concentration from RI water upstream of the floodplain to FP waters exiting the floodplain (**Table 2.2 b**). The overall decrease or retention of DIP (or MRP) is in agreement with previous studies that have assessed phosphate removal from incident floodwaters in riparian systems in the southeastern US (Richardson et al. 1988, Brinson 1993, Lockaby and Walbridge 1998, Darke and Walbridge 2000, Bruland and Richardson 2004, Hupp et al. 2005). This decrease could be due in part to adsorption and precipitation reactions with mineral forms of Al and/or Fe, or to the complexation and precipitation of DIP with organic matter in solution, via OM – metal (Al, Fe) – P complexation (Axt and Walbridge 1999, Darke and Walbridge 2000, Hogan et al. 2004, Hupp et al. 2005). Dissolved Al decreased (up to 57 fold) in FP compared to RI floodwaters in AL systems (**Figure 2.6 b**), and DOC concentrations were higher in the FP than in RI waters in AL systems (**Table 2.2 a**), possibly the result of solubilization associated with flood-induced DOC entrainment in the water column (Reddy et al. 1999, Neatrour et al. 2004, Kleeberg et al. 2007). Walbridge and Huang (unpublished data), observed similar decreases in DIP occurring concomitantly with increases in DOC content, which was significantly positively correlated with organically-bound Al ($r = 0.96$, $P < 0.0001$) in inflow and outflow floodwater that were collected in AL systems during the same flooding events. In combination, these trends -- the increase in DOC concentrations, the significant decrease in Al concentration, and the significant decrease in DIP -- suggest OM – Al – P complexation (Cheng et al. 2004, Giesler et al. 2005, Jensen et al. 2005, Guan et al. 2006, Novak and Watts 2006, de Vicente et al. 2008) as a plausible mechanism

of inorganic P transformation during flooding events in AL systems (Novak and Watts 2006). Although similar trends were not observed in FP waters within BW systems, Al and Fe concentrations were significantly higher in BW compared to AL systems, and higher DOC content in BW RI compared to FP waters, suggesting that DIP sorption/precipitation reactions with Al/Fe cations and/or complexation with OM-Al/Fe may have occurred in the floodwater column prior to entering BW floodplains.

2.4.1.2 ^{31}P NMR Detected Inorganic (Condensed) and Organic P Forms in Floodwaters

Floodplain forests are frequently thought of as sinks for inorganic P and sources of organic P pools (Richardson et al. 1988, Brinson 1993, Lockaby and Walbridge 1998, Darke and Walbridge 2000, Bruland and Richardson 2004, Hupp et al. 2005), yet previous studies have estimated organic P pools using traditional methods (**Figure 2.1 a**) that are likely to overestimate of the amount of “true” organic P found in floodwaters (Darke and Walbridge 1994, McKelvie et al. 1995, Benitez-Nelson et al. 2007), while providing little information on P speciation. In addition to the chemical transformation of P with OM via metal (Al and/or Fe) ligands, biotic transformation of inorganic P to organic P could act as an additional line of defense for reducing DIP loads to downstream aquatic ecosystems. The majority of the DIP pool and most of the P extracted from these water samples was orthophosphate (**Table 2.2 b, Figure 2.3 – 2.4**). This is the simplest ionic form of P to occur naturally, hence dissolved orthophosphate is the most readily bioavailable P form (McKelvie et al. 1995, Paytan et al. 2003, Cade-Menun 2005a, He et al. 2006, McDowell and Koopmans 2006, Bourke et al. 2008). Both DIP in general and dissolved orthophosphate specifically decreased significantly in FP as

compared to RI waters in both FF types (**Figure 2.7 b**), averaging 26 and 17 % of river dissolved orthophosphate concentrations in AL and BW floodplains, respectively.

Dissolved pyrophosphates on average ranged from 1.6 to 8.6 % of total extracted P (**Table 2.2 b**) and were significantly higher in FP than in RI waters in AL and BW systems (**Figure 2.7 c**). Pyrophosphates (**Figure 2.2 b**), which are the smallest chemical form of polyphosphates, are common in soils (Makarov 1998, Cardoso et al. 2003a, Chiu et al. 2005, McDowell et al. 2007a, Dou et al. 2009), marine (Carman et al. 2000, Cade-Menun et al. 2005, Ahlgren et al. 2006b) and freshwater sediments (Hupfer et al. 2004, Ahlgren et al. 2006a, Reitzel et al. 2006, Ahlgren et al. 2007, Liu et al. 2009), estuarine sediments (Sundareshwar et al. 2001), and marine sediment porewater and water column (Kolowitz et al. 2001, Paytan et al. 2003). Pyrophosphates are produced by microbial activity (Gerlitz and Gerlitz 1997, Watts et al. 2002, Makarov et al. 2005) and recent evidence indicates a higher concentration of pyrophosphate in fungal (endophytic and saprotrophic) vs. bacterial biomass in soil isolates (Makarov et al. 2005, Bunemann et al. 2008). Soil drying and rewetting, such as can occur during periodic flooding events, can lyse microbial cells due to osmotic shock upon rapid rewetting resulting in the release of water-extractable P forms, including pyrophosphates (Turner et al. 2003c). Significant and consistently higher dissolved pyrophosphate concentration in FP waters across AL and BW systems may be the first specific identification of the primary mechanisms associated with biotic uptake of DIP and subsequent release of condensed pyrophosphates in FFs. Pyrophosphates are utilized by both plants and soil microbes, through the use of pyrophosphatases (Sundareshwar et al. 2001, Cheesman et al. 2010), but the presence of this enzyme or the ability to hydrolyze pyrophosphate extracellularly in the water column has only been characterized in two marine dinoflagellates

(Oh et al. 2002).

The overall concentration decrease in DIP forms, particularly with respect to a decrease in readily available orthophosphate concentrations along the flow path from RI to FP floodwaters, occurred concomitantly with significant increases in the sum of DOP forms in FP compared to RI waters during flooding events in both AL and BW floodplain forests (**Figure 2.7 d**). Orthophosphate monoesters comprised the majority of this DOP pool in floodwaters, which also increased significantly in FP vs. RI waters (**Figure 2.7 e**). Orthophosphate monoesters are biologically synthesized compounds (Makarov et al. 2005, Bunemann et al. 2008, Koukol et al. 2008), and are the most common organic P compounds found in soils in both managed (Cardoso et al. 2003b, Toor et al. 2005, McDowell and Koopmans 2006, Dou et al. 2009) and natural ecosystems (Cade-Menun et al. 2000, Turner et al. 2004, McDowell et al. 2007b), and in both marine particulates (Clark et al. 1999, Carman et al. 2000, Paytan et al. 2003, Cade-Menun et al. 2005) and freshwater sediments (Turner and Newman 2005, Reitzel et al. 2006, Liu et al. 2009, Zhang et al. 2009). Orthophosphate monoesters include several compound types, such as sugar phosphates (e.g. glycerophosphate), mononucleotides, and inositol phosphates of varying degrees of phosphorylation (e.g. *scyllo*-IP). The majority of the dissolved orthophosphate monoesters present (averaging 22.8 to 32.8 % of extracted P in RI and FP waters, respectively) were in the undistinguished “other” category (**Table 2.2 b**). These may represent glucose phosphate and mononucleotide degradation products of RNA and/or phospholipids (Turner et al. 2003b), and were consistently higher in FP than in RI waters in both AL and BW systems. Glycerophosphate and *scyllo*-IP were present, but in small proportions (< 3 % of extracted P) and in only some water samples (**Table 2.2 b**), whereas the average phytate concentrations ranged

from 1.7 to 7.2 % of extracted P and were significantly higher in FP than in RI waters in both AL and BW floodplain forests (**Figure 2.7 f**). Inositol phosphates are complex molecules composed of phosphate groups attached to a six carbon ring, and in terrestrial environments they are synthesized by plants and accumulate in soils because of their high charge density that form strong interactions with clays and Al/Fe minerals (Turner et al. 2002, Turner and Weckström 2008). Although orthophosphate is released from the dephosphorylation of inositol phosphates by plant and microbially produced extracellular phytases in soils, inositol phosphates are less prone to hydrolysis in freshwater ecosystems which may be due to the low pH requirement (pH 2.5 – 5) for phytase activity (Selig et al. 2002, Turner et al. 2002, Turner and Weckström 2008).

Dissolved orthophosphate diesters, predominantly DNA, were present in most samples (**Table 2.2 b**), and were generally higher in FP than in RI waters at both AL and BW floodplain forests. The actual amount of diester P forms may have been underestimated, because some peaks detected in the monester region, such as glycerophosphates, are degradation products of phospholipids, which are orthophosphate diesters (Buenemann et al. 2008, Doolette et al. 2009) and were also generally higher in FP waters at both FF types.

Total PP recovery following NaOH-EDTA extraction averaged 67 % in all water samples, whereas TDP recovery averaged 41 % and was similar to the recovery of Ca (49 %) in these waters. The range of recoveries was comparable to values obtained for TP (60 – 90 %) (Minear 1972) in lake water, DOP (25 – 50 %), and POP (23 – 32 %) in marine water samples (Kolowith et al. 2001). It is likely that the larger the volume of extractant used for the filters increased total P recovery of PP relative to DP. It is possible that the EDTA was saturated with cations with the

smaller volume of extractant used for DP, limiting the recovery of DP and Ca. Although the total P was not recovered from either PP or DP, the extracted P is expected to be representative of all the P forms in the sample, with no selected extraction (Paytan et al. 2003).

2.4.2 Particulate P Forms in Floodwaters

Previous research in AL floodplain forests has suggested that one mechanism for P retention involves sedimentation or the precipitation of suspended material (primarily POP and PIP adsorbed to mineral sediments) from the water column (Kleiss 1996, Craft and Casey 2000, Hupp et al. 2005, Benitez-Nelson et al. 2007, Brunet and Astin 2008). I had hypothesized PP pools to decrease as upstream (RI) floodwaters flowed over the floodplain surface and exited at the downstream (FP) location, suggesting PP removal from the water column by sedimentation, but differences in PP in RI and FP waters were statistically indistinguishable across both AL and BW systems. ANOVA results of differences in TPP concentrations between RI and FP waters across FFs did not show significant differences by floodplain type (AL and BW systems), location (RI and FP), or the interaction term (floodplain type $\times$ RI/FP location) (**Appendix Table 2.1 c**). The same was true for the proportion of total P in colloidal form, which did not differ by floodplain type ($F = 0.105$, $p = 0.756$), RI/FP location ($F = 0.207$, $p = 0.665$), or an interaction between the two factors ($F = 2.990$, $p = 0.134$). Additionally, the sum of NMR-visible PIP and POP pools did not differ between sampling locations (RI versus FP) within FFs, or across AL and BW floodplain systems (**Appendix Table 2.1 c**). These observations are consistent with Venterink et al. (2003), who concluded that the flow velocity of a tributary of the river Rhine (Muyzer et al.) in the Netherlands was too high for PP removal compared to the relatively slower

flow rate of the river Ijssel. The efficiency of PP removal from the water column depends on the contact area between water and soil, the flow velocity of the floodwaters over floodplain surface, and the size of the suspended particles. I collected and analyzed floodwaters at peak discharge onto the FFs, where the flow velocity of floodwaters may have been too high for PP precipitation to occur as floodwaters transited the FFs. In AL floodplains, average PP increased (though not significantly) as RI ($52.9 \pm 10.0 \mu\text{g L}^{-1}$) waters entered the FP ($73.9 \pm 18.9 \mu\text{g L}^{-1}$). This may be typical of rivers with high discharge rates, such as AL rivers. In contrast, average PP concentrations showed the opposite trend (i.e., were higher in RI than in FP waters) in BW floodplain forests (**Table 2.2 c**). Blackwater systems have smaller drainage areas than AL systems, with lower gradient flows that have limited potential to entrain or erode sediments. In tributaries of the Mississippi river, Alexander et al. (2000) found that nutrient retention increased with decreasing water depth. Shallow waters with maximum contact between water and floodplain surface are favorable for sedimentation or the removal PP from the water column (Johnston 1991, Brunet and Astin 1998). Floodwater energy influences the balance between sedimentation (retention) vs. sediment entrainment – the scouring of PP from the FFs surface during peak discharge (peak) or high velocity floodwaters. The time required for processing (filtering and lyophilizing) 10 L of water per location (within the analytical parameters assigned for P analysis) or the relatively high costs of NMR analysis on extracts limits the number of floodwater samples that can be collected and analyzed throughout the duration of the flooding events. Measuring fluxes in P pools over the course of the flooding events or the hydroperiod on the floodplain, particularly during the falling limb of the flood stage height when floodwater velocity is relatively slower, may capture higher amounts of precipitating PP adsorbed to suspended mineral sediments (Noe and Hupp 2007, 2009). Finally, differences between

dissolved and particulate P fractions were assessed at each location within each FF to determine the origin of NMR-visible P forms. The ANOVA results of the differences in dissolved and particulate pyrophosphate at each sampling location were statistically significant and higher in FP (3.7 ± 1.7 %) than in RI (-2.8 ± 1.4 %) waters (RI/FP location effect: $F = 15.284$, $p = 0.008$) at both AL and BW floodplain types (data not shown). Similarly, differences in dissolved and particulate phytate was significantly higher in FP (0.8 ± 2.3 %) compared to RI (-3.7 ± 1.8 %) waters (RI/FP location effect: $F = 9.230$, $p = 0.023$) at both floodplain forest types. Higher dissolved pyrophosphate and phytate content in FP waters may indicate that these compounds are either transported from terrestrial material (phytate) or formed by aquatic organisms (pyrophosphate).

Overall, a significant decrease in total DIP pools and a concurrent increase in DOP forms in inflow (RI) versus outflow (FP) waters suggest increased biogeochemical reactivity of DIP in the floodplain. Higher total DIP concentrations in floodwaters entering the floodplain, along with the net increase in biogenic DOP forms in outflow waters, is consistent with the theory that floodplain forests regulate river water nutrient concentrations through the biotic transformation of nutrients during flooding events (Brunet and Astin, 1998; Lockaby and Walbridge 1998).

2.5 CONCLUSIONS

Solution ^{31}P -NMR spectroscopy is routinely used to identify P forms in soils and sediments, including orthophosphate monoesters, orthophosphate diesters, phosphonates, polyphosphates and pyrophosphates. However, only a few have been conducted for freshwaters (Nanny and Minear 1994, 1997, Selig et al. 2002, Cade-Menun et al. 2006). This study demonstrates the feasibility of ^{31}P -NMR as a method for characterizing and quantifying forms of inorganic and organic P in freshwater samples, and that readily available orthophosphate is converted to less available organic P forms in floodwaters, suggesting biological transformation of inorganic P to organic P in FF ecosystems. Although the conceptual model of FFs is that they reduce TP concentrations from riverine inputs, some floodplains exhibit the opposite trend and are sources of TP (and/or DIP or MRP) (Bonetto et al. 1994, Villar et al. 1998, Carlyle and Hill 2001, Noe and Hupp 2007, Tian and Zhou 2007, Loeb et al. 2008). In this study, TP concentrations were similar in both floodplain types, and although I had expected (in my first hypothesis) TP to decrease in RI vs FP waters, results support the alternative hypothesis where TP (the sum of DP and PP) pools were neither an overall sink nor source from AL and BW floodplains.

Although TDP did not differ between RI and FP locations in the BW systems, there was a significant decrease in TDP in FP ($10.8 \pm 2.7 \mu\text{g L}^{-1}$) compared to RI ($22.8 \pm 5.4 \mu\text{g L}^{-1}$) waters (**Table 2.2 a**) in the AL systems (**Appendix Table 2.1 a** and **Figure 2.6 a**). In AL systems that are characteristically larger in drainage area compared to BW floodplains, TDP inputs decreased as river waters entered the floodplain during flooding events, contributing to an average 53 % reduction in river TDP load. Alluvial FFs were net sinks for reactive DIP forms (retaining 21 %

of total floodwater DIP inputs). The increase in DOC and dissolved Al content, and the accompanied decrease in DIP forms, may suggest OM – metal (Al, Fe) – P complexation as one possible mechanism of inorganic P removal from the water column.

During flooding events, AL and BW floodplains transform 21 and 11 % of the sum of NMR-visible DIP forms, respectively. Of these DIP forms, the most readily bioavailable – orthophosphate in river waters was transformed at rates of 26 and 17 % within AL and BW floodplains, respectively. I had expected (in my second hypothesis) that P pools in river waters flowing into FFs were dominated by inorganic P forms, while the P in outflow waters was predominantly organic P. This conversion of DIP forms (e.g. orthophosphate) to dissolved condensed inorganic P forms (pyrophosphates) and low molecular weight DOP compounds (e.g. orthophosphate monoesters) suggests biological P transformation, a second important process occurring during flooding events, and likely leads to less available P forms downstream of these FFs.

In my third hypothesis, I expected AL rivers that carry higher sediment loads, to retain larger amounts of PP within the floodplain, but in both AL and BW systems, PP in RI and FP waters, and the sum of NMR-visible PIP and POP pools in these waters did not differ significantly between sampling locations. Because samples were collected during peak flood discharge, flow velocities may have been too high for PP precipitation to occur. Additional measurements over the course of a flooding event, as floodwater velocity declines, might capture larger amounts of precipitating PP. Nevertheless, NMR results clearly indicate that FFs in the southeastern US

provide an important ecosystem service, capable of strong P transformation potential, which underlines the benefits of maintaining these wetlands. This knowledge will be critical to assessing the sustainability of FF ecosystem services, as well as replicating them through wetland creation or restoration.

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Figure 2.1: Operationally defined P forms in freshwater aquatic systems. Figure adapted from (Worsfold et al. 2008).

TP: total P	TRP: total reactive P
TOP: total organic P	TDP: total dissolved P
TPP: total particulate P	DRP: dissolved reactive P
DOP: dissolved organic P	POP: particulate organic P
DIP: dissolved inorganic P	PIP: particulate inorganic P

Figure 2.2: Examples and structures of ^{31}P -NMR resolved inorganic and organic P forms. Figure adapted from (Cade-Menun 2005a, Worsfold et al. 2008) .

Name (abbreviation)	General Structure	Examples
a. Orthophosphate (Ortho – P)	$\begin{array}{c} \text{OH} \\ \\ \text{HO}-\text{P}-\text{OH} \\ \\ \text{O} \end{array}$	
b. Pyrophosphate (Pyro – P)	$\begin{array}{c} \text{O} \quad \quad \text{O} \\ \quad \quad \\ \text{HO}-\text{P}-\text{O}-\text{P}-\text{OH} \\ \quad \quad \\ \text{OH} \quad \quad \text{OH} \end{array}$	
c. Inorganic Polyphosphates (Poly – P)	$\begin{array}{c} \text{O} \quad \quad \text{O} \quad \quad \text{O} \\ \quad \quad \quad \quad \\ \text{HO}-\text{P}-\text{O}-\left[\text{P}-\text{O}-\text{P} \right]_X-\text{P}-\text{OH} \\ \quad \quad \quad \quad \\ \text{OH} \quad \quad \text{OH} \quad \quad \text{OH} \end{array}$	Organic polyphosphates such as Adenosine 5-phosphate ($\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_{13}\text{P}_3$) may also occur.
d. Orthophosphate monoesters (Mono – P)	$\begin{array}{c} \text{OH} \\ \\ \text{HO}-\text{P}-\text{O}-\text{R} \\ \\ \text{O} \end{array}$	<p>Phytate</p>
e. Orthophosphate diesters (Diester – P)	$\begin{array}{c} \text{OH} \\ \\ \text{HO}-\text{P}-\text{O}-\text{R}_1 \\ \\ \text{O} \\ \\ \text{R}_2 \end{array}$	Deoxyribonucleic acid (DNA) ($\text{C}_2\text{H}_8\text{NO}_4\text{P}$)
f. Phosphonates	$\begin{array}{c} \text{OH} \\ \\ \text{HO}-\text{P}-\text{R} \\ \\ \text{O} \end{array}$	$\begin{array}{c} \text{OH} \\ \\ \text{HO}-\text{P}-\text{CH}_2-\text{CH}_2-\text{H}_2\text{N} \\ \\ \text{O} \end{array}$ <p>2-Aminoethyl phosphoric acid</p>

Figure 2.3: Solution ^{31}P nuclear magnetic resonance spectra of water samples collected in an alluvial system at the Pee Dee River, SC. Panels represent: **a**, particulate P forms and; **b**, dissolved P forms in water samples collected from the floodplain; **c**, particulate P forms and; **d**, dissolved P forms in water samples collected from the river inlet. Spectra were generated by B. J. Cade-Menun on a Varian ^{UNITY} INOVA 600 MHz spectrometer equipped with a 10-mm broadband probe. Parameters were: temperature of 20°C; using a 90° pulse; 0.68 s acquisition time; 4.32 s relaxation delay; 12 Hz spinning; and an external H_3PO_4 standard, collecting 5800 scans for particulate and 8500 scans for dissolved P samples.

Alluvial system; Pee Dee River, SC

Figure 2.4: Solution ^{31}P nuclear magnetic resonance spectra of water samples collected in a blackwater system at the Blackwater River, NC. Panels represent: **a**, particulate P forms and; **b**, dissolved P forms in water samples collected from the floodplain; **c**, particulate P forms and; **d**, dissolved P forms in water samples collected from the river inlet. Spectra were generated by B. J. Cade-Menun on a Varian ^{UNITY} INOVA 600 MHz spectrometer equipped with a 10-mm broadband probe. Parameters were: temperature of 20°C; using a 90° pulse; 0.68 s acquisition time; 4.32 s relaxation delay; 12 Hz spinning; and an external H_3PO_4 standard, collecting 5800 scans for particulate and 8500 scans for dissolved P samples.

Blackwater system; Black River, NC

Figure 2.5: Solution ^{31}P nuclear magnetic resonance spectra of a water sample collected at the Satilla River, GA with the identified particulate P forms (from L to R): Orthophosphate, orthophosphate monoesters (phytate, glycerophosphate (GP), *scyllo*-inositol phosphate), orthophosphate diesters (including DNA), and inorganic pyrophosphate. Spectra were generated by B. J. Cade-Menun on a Varian ^{UNITY} INOVA 600 MHz spectrometer equipped with a 10-mm broadband probe. Parameters were: temperature of 20°C; using a 90° pulse; 0.68 s acquisition time; 4.32 s relaxation delay; 12 Hz spinning; and an external H_3PO_4 standard, collecting 5800 scans for particulate P samples. The large orthophosphate peak was truncated so that smaller peaks could be visualized.

Figure 2.6: Mean ($\pm$ SE) of the a) proportion of total P in dissolved form, b) Al, c) Fe, and c) particulate phytate concentrations in alluvial (AL) and blackwater (BW) river inlets (RI) and floodplain (FP) waters. A 2-way ANOVA with floodplain type (AL vs. BW) and location (RI vs. FP) showed significant main effects in floodplain type and an interaction between the two factors. Bars marked with the same upper-case letters are not significantly different from each other ($P < 0.05$, Tukeys HSD test for *a posteriori* comparison). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 2.1**

Figure 2.7: Mean ($\pm$ SE) of NMR visible dissolved inorganic and organic P: a) sum of dissolved inorganic P, b) dissolved orthophosphate, and c) dissolved inorganic pyrophosphate, d) sum of dissolved organic phosphorus, e) sum of dissolved orthophosphate monoesters, and f) dissolved phytate concentrations in alluvial (AL) and blackwater (BW) river inlets (RI) versus floodplain (FP) waters. A 2-way ANOVA with floodplain type (AL vs. BW) and location (RI vs. FP) showed significant main effect by RI/FP location ($P < 0.05$, students t-test for *a posteriori* comparison). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 2.1**

Figure 2.8: Mean ($\pm$ SE) of the a) sum of particulate orthophosphate monoesters and b) particulate *scyllo*-inositol phosphates in alluvial (AL) and blackwater (BW) river inlets (RI) and floodplain (FP) waters. A 2-way ANOVA with floodplain type (AL vs. BW) and location (RI vs. FP) showed significant main effect by floodplain type ($P < 0.05$, students t-test for *a posteriori* comparison). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 2.1**

Table 2.1: Locations and characteristics of the river systems in the southeastern U.S. Stream flow River data from USGS gauging stations located near each study site.

River type	Site	Latitude	Longitude	Drainage area (km ²)	Mean daily stream flow (m ³ s ⁻¹)	TP range (µg L ⁻¹)	TP sampling date
Alluvial	Nottoway River, VA	36° 46' N	77° 09' W	3,732	410	67.0 – 129.0	9/1999
--- " ---	Pee Dee River, SC	34° 12' N	79° 32' W	22,870	2,995	60.0 – 160.0	1/1995 – 9/1995
--- " ---	Oconee River, GA	32° 32' N	82° 53' W	11,396	1,474	60.0	11/1976
--- " ---	Ocmulgee River, GA	31° 55' N	82° 40' W	13,416	1,665	30.0 – 70.0	3/2005 – 7/2005
Mean (SE)				12,854 (3,937)*	1,636 (530)*	75.6 (12.6)	
Blackwater	Black River, NC	34° 45' N	78° 18' W	1,751	242	62.0 – 332.0	5/2009 – 12/2009
--- " ---	Drowning Creek, NC	35° 03' N	79° 29' W	474	76	n.a.	
--- " ---	Big Swamp, NC	34° 42' N	78° 50' W	593	67	n.a.	
--- " ---	Satilla River, GA	31° 14' N	82° 19' W	3,108	311	50.0	7/1974
Mean (SE)				1,482 (614)	174 (61)	109 (59.0)	

* Indicates significant differences between alluvial and blackwater rivers ($p \leq 0.05$).

Table 2.2: Distribution of a) dissolved and particulate total P, MRP, C, Al, Fe, pH, and conductivity measured by chemical analysis, b) NMR visible dissolved P forms, and c) NMR visible particulate P forms in individual river inlet (RI) and floodplain (FP) samples collected at alluvial and blackwater systems in the southeastern U.S. * Indicates significant differences between alluvial and blackwater rivers (RI) for measured components ($p \leq 0.05$) using a two-sample t-test analysis. For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 2.1**. Abbreviations: TDP, total dissolved P; MRP, molybdate reactive P; C, carbon; Al, aluminum; Fe, iron; Cond, conductivity; TPP, total particulate P; orthoP, orthophosphate; pyroP, pyrophosphate; polyP, polyphosphate; PA, phytate; *scyllo*-IP, *scyllo*-inositol phosphate; GP, glycerophosphate; DNA, deoxyribonucleic acid; phon, phosphonate.

Table 2.2 a: Dissolved and particulate Total P, MRP, C, Al, Fe, as well as pH and conductivity.

		Dissolved							Particulate	
Alluvial systems		TDP ($\mu\text{g L}^{-1}$)	MRP ($\mu\text{g L}^{-1}$)	C ($\mu\text{g L}^{-1}$)	Al ($\mu\text{g L}^{-1}$)	Fe ($\mu\text{g L}^{-1}$)	pH	Cond.	TPP ($\mu\text{g L}^{-1}$)	C ($\mu\text{g L}^{-1}$)
Nottoway River, VA	RI	11.1	7.1	9600.0	5146.0	24.3	5.9	25.5	48.6	5340.0
	FP	6.6	6.4	2573.0	106.4	6.6	6.2	27.8	52.9	5580.0
Pee Dee River, SC	RI	34.0	21.0	5170.0	4.9	65.4	7.0	90.3	64.7	3300.0
	FP	11.0	14.2	8197.0	0.0	19.3	6.6	69.3	103.4	16570.0
Oconee River, GA	RI	29.8	22.0	3523.0	4012.0	23.7	6.9	71.1	71.5	3820.0
	FP	18.3	6.8	14050.0	90.5	34.7	6.6	66.5	107.9	7800.0
Ocmulgee River, GA	RI	16.3	5.8	4468.0	2139.0	10.8	6.9	36.9	26.8	2830.0
	FP	7.2	5.5	13340.0	0.9	22.3	7.0	39.1	31.2	1260.0
<i>Mean (S. E.)</i>	RI	<i>22.8</i> <i>(5.4)</i>	<i>14.0</i> <i>(4.4)</i>	<i>5,690.2</i> <i>(1,346.2)</i>	<i>2,825.5</i> <i>(1,126.2)</i>	<i>31.1</i> <i>(11.9)</i>	<i>6.4*</i> <i>(0.6)</i>	<i>56.0</i> <i>(15.0)</i>	<i>52.9</i> <i>(10.0)</i>	<i>3,823.3</i> <i>(770.4)</i>
	FP	<i>10.8</i> <i>(2.7)</i>	<i>8.2</i> <i>(2.0)</i>	<i>9,540.0</i> <i>(2,663.4)</i>	<i>49.5</i> <i>(28.5)</i>	<i>20.7</i> <i>(5.8)</i>	<i>6.5</i> <i>(0.2)</i>	<i>50.7</i> <i>(10.2)</i>	<i>73.9</i> <i>(18.9)</i>	<i>7,803.3</i> <i>(4,557.3)</i>
Blackwater systems										
Black River, NC	RI	97.7	25.0	23210.0	64300.0	389.7	6.0	79.0	119.3	14300.0
	FP	127.4	10.8	11730.0	88310.0	382.6	5.3	48.5	60.2	9880.0
Drowning Creek, NC	RI	8.0	6.3	101700.0	73480.0	305.6	5.3	18.6	10.7	2650.0
	FP	8.8	5.8	28020.0	52970.0	382.6	5.4	21.9	5.5	7970.0
Big Swamp, NC	RI	17.2	7.0	9983.0	4293.0	36.9	5.9	59.7	23.4	22730.0
	FP	42.7	5.9	17280.0	7323.0	84.0	5.3	72.4	25.4	23040.0
Satilla River, GA	RI	43.2	22.6	19630.0	7903.0	490.4	5.7	40.9	25.4	7640.0
	FP	40.3	22.2	8207.0	4870.0	176.6	5.7	42.2	30.7	5590.0
<i>Mean (S. E.)</i>	RI	<i>41.5</i> <i>(20.2)</i>	<i>15.2</i> <i>(5.0)</i>	<i>38,630.8</i> <i>(21,207.8)</i>	<i>37,494.0</i> <i>(18,238.0)</i>	<i>305.7*</i> <i>(97.2)</i>	<i>5.6</i> <i>(0.2)</i>	<i>49.6</i> <i>(12.9)</i>	<i>44.7</i> <i>(25.1)</i>	<i>11,830.0</i> <i>(4,346.8)</i>
	FP	<i>54.8</i> <i>(25.4)</i>	<i>11.2</i> <i>(3.8)</i>	<i>16,309.3</i> <i>(4,327.2)</i>	<i>38,368.3</i> <i>(19,986.1)</i>	<i>256.5</i> <i>(75.2)</i>	<i>5.4</i> <i>(0.1)</i>	<i>46.2</i> <i>(10.4)</i>	<i>30.4</i> <i>(11.3)</i>	<i>11,620.0</i> <i>(3,906.5)</i>

Table 2.2 b: NMR visible dissolved P forms.

Dissolved P forms (%)		Inorganic P				Orthophosphate monoesters					Orthophosphate diesters				
		orthoP	pyroP	polyP	ΣP_i	PA	Scyllo-IP	GP	Other mono	$\Sigma mono$	DNA	other di	Σdi	phon	ΣP_o
Alluvial systems															
Nottoway River, VA	RI	72.8	0.0	0.0	72.8	0.0	0.0	0.0	24.2	24.2	3.0	0.0	3.0	0.0	27.2
	FP	57.6	3.4	0.0	61.0	0.0	0.0	0.0	37.2	37.3	1.7	0.0	1.7	0.0	39.0
Pee Dee River, SC	RI	77.5	0.0	0.0	77.5	0.0	0.0	0.9	20.4	21.3	1.2	0.0	1.2	0.0	22.5
	FP	58.3	7.8	0.0	66.1	4.5	0.0	0.7	26.8	32.0	1.9	0.0	1.9	0.0	33.9
Oconee River, GA	RI	84.6	3.1	0.0	87.7	2.1	0.7	0.0	8.5	11.3	1.0	0.0	1.0	0.0	12.3
	FP	51.0	4.8	0.0	55.8	4.9	1.6	1.6	33.2	41.3	1.9	1.0	2.9	0.0	44.2
Ocmulgee River, GA	RI	49.4	3.4	0.0	52.8	4.8	1.6	1.6	38.0	46.0	0.0	1.2	1.2	0.0	47.2
	FP	44.7	3.5	0.0	48.2	11.3	0.0	2.8	34.0	48.2	2.4	1.2	3.6	0.0	51.8
Mean (S. E.)	RI	71.1	1.6	0.0	72.7	1.7	0.6	0.6	22.8	25.7	1.3	0.3	1.6	0.0	27.3
		(7.6)	(0.9)	(0.0)	(7.3)	(1.1)	(0.4)	(0.4)	(6.1)	(7.3)	(0.6)	(0.3)	(0.5)	(0.0)	(7.3)
	FP	52.9	4.9	0.0	57.8	5.2	0.4	1.3	32.8	39.7	2.0	0.6	2.5	0.0	42.2
		(3.2)	(1.0)	(0.0)	(3.8)	(2.3)	(0.4)	(0.6)	(2.2)	(3.4)	(0.1)	(0.3)	(0.4)	(0.0)	(3.8)
Blackwater systems															
Black River, NC	RI	77.2	5.1	0.8	83.1	2.8	1.0	1.0	10.5	15.3	0.8	0.0	0.8	0.8	16.9
	FP	55.0	11.6	0.0	66.6	6.1	1.0	1.0	21.4	29.5	1.6	2.3	3.9	0.0	33.4
Drowning Creek, NC	RI	42.7	2.7	0.0	45.4	0.0	0.0	0.0	50.6	50.6	2.7	1.3	4.0	0.0	54.6
	FP	46.1	5.6	0.0	51.7	2.7	0.9	0.0	39.1	42.7	5.6	0.0	5.6	0.0	48.3
Big Swamp, NC	RI	46.3	3.2	0.0	49.5	11.0	0.0	1.8	37.5	50.5	0.0	0.0	0.0	0.0	50.5
	FP	26.2	3.6	0.0	29.8	17.7	0.3	2.0	46.3	69.0	1.2	0.0	1.2	0.0	70.2
Satilla River, GA	RI	74.6	10.1	1.3	86.0	0.0	0.0	0.0	11.4	11.4	1.3	1.3	2.6	0.0	14.0
	FP	73.5	13.6	0.0	87.1	2.4	0.8	0.8	8.0	12.0	0.9	0.0	0.9	0.0	12.9
Mean (S. E.)	RI	60.2	5.3	0.5	66.0	3.5	0.3	0.7	27.5	32.0	1.2	0.7	1.9	0.2	34.0
		(9.1)	(1.7)	(0.3)	(10.8)	(2.6)	(0.3)	(0.4)	(9.9)	(10.8)	(0.6)	(0.4)	(0.9)	(0.2)	(10.8)
	FP	50.2	8.6	0.0	58.8	7.2	1.4	1.0	28.7	38.3	2.3	0.6	2.9	0.0	41.2
		(9.8)	(2.4)	(0.0)	(12.1)	(3.6)	(0.5)	(0.4)	(8.7)	(12.0)	(1.1)	(0.6)	(1.1)	(0.0)	(12.1)

Table 2.2 c: NMR visible particulate P forms.

Particulate P forms (%)		Inorganic P				Orthophosphate monoesters					Orthophosphate diesters				
		orthoP	pyroP	polyP	ΣP_i	PA	scyllo-IP	GP	other mono	$\Sigma mono$	DNA	other di	Σdi	phon	ΣP_o
Alluvial systems															
Nottoway River, VA	RI	81.9	1.7	0.0	83.6	5.2	1.7	1.7	7.8	16.4	0.0	0.0	0.0	0.0	16.4
	FP	83.2	1.8	0.9	85.9	2.6	1.8	7.9	0.9	13.2	0.9	0.0	0.9	0.0	14.1
Pee Dee River, SC	RI	78.2	2.5	0.0	80.7	8.8	1.9	0.9	6.9	18.5	0.8	0.0	0.8	0.0	19.3
	FP	69.9	3.5	0.0	73.4	5.5	1.8	1.0	16.5	24.8	0.9	0.0	0.9	0.9	26.6
Oconee River, GA	RI	71.9	7.5	0.0	79.4	5.8	1.0	1.0	8.7	16.5	4.1	0.0	4.1	0.0	20.6
	FP	68.3	4.8	0.0	73.1	5.7	1.0	1.0	15.1	22.8	4.1	0.0	4.1	0.0	26.9
Ocmulgee River, GA	RI	78.0	1.6	0.0	79.6	4.5	2.2	1.5	10.5	18.8	0.8	0.0	0.8	0.8	20.4
	FP	75.0	3.0	0.0	78.0	3.4	2.2	1.1	11.3	18.0	3.0	1.0	4.0	0.0	22.0
Mean (S. E.)	RI	77.5 (2.1)	3.3 (1.4)	0.0 (0.0)	80.8 (1.0)	6.1 (0.9)	1.7 (0.3)	1.3 (0.2)	8.5 (0.8)	17.6 (0.6)	1.4 (0.9)	0.0 (0.0)	1.4 (0.9)	0.2 (0.2)	19.2 (1.0)
	FP	74.1 (3.4)	3.3 (0.6)	0.2 (0.2)	77.6 (3.0)	4.3 (0.8)	1.7 (0.3)	2.8 (1.7)	11.0 (3.5)	19.7 (2.6)	2.2 (0.8)	0.3 (0.3)	2.5 (0.9)	0.2 (0.2)	22.4 (3.0)
Blackwater systems															
Black River, NC	RI	82.1	1.9	0.0	84.0	5.5	1.9	7.5	1.0	16.0	0.0	0.0	0.0	0.0	16.0
	FP	68.1	0.9	0.0	69.0	5.2	3.4	19.8	1.7	30.1	0.9	0.0	0.9	0.0	31.0
Drowning Creek, NC	RI	30.0	6.0	0.0	36.0	4.3	7.2	2.2	35.3	49.0	12.0	3.0	15.0	0.0	64.0
	FP	37.8	5.1	0.0	42.9	5.2	5.2	1.8	34.7	46.9	8.2	2.0	10.2	0.0	57.1
Big Swamp, NC	RI	28.1	5.6	0.0	33.7	5.2	3.5	2.6	46.0	57.3	7.9	1.1	9.0	0.0	66.3
	FP	53.3	3.7	0.0	57.0	5.0	4.2	1.7	29.3	40.2	2.8	0.0	2.8	0.0	43.0
Satilla River, GA	RI	68.0	3.0	0.0	71.0	10.9	3.6	2.6	10.9	28.0	1.0	0.0	1.0	0.0	29.0
	FP	71.1	1.7	0.0	72.8	10.5	5.3	1.7	8.8	26.3	0.9	0.0	0.9	0.0	27.2
Mean (S. E.)	RI	52.1 (13.6)	4.1 (1.0)	0.0 (0.0)	56.2 (12.6)	6.5 (1.5)	4.1* (1.1)	3.7* (1.3)	23.3 (10.5)	37.6 (9.5)	5.2 (2.9)	1.0 (0.7)	6.3 (3.5)	0.0 (0.0)	43.8 (12.6)
	FP	57.6 (7.7)	2.9 (1.0)	0.0 (0.0)	60.4 (6.7)	6.5 (1.3)	4.5 (0.5)	6.3 (4.5)	18.6 (7.9)	35.9 (4.7)	3.2 (1.7)	0.5 (0.5)	3.7 (2.2)	0.0 (0.0)	39.6 (6.7)

CHAPTER 3

Retention and Transformation of $^{32}\text{PO}_4^{3-}$ During Simulated Flooding of Floodplain Forest Soil Mesocosms

ABSTRACT

Floodplain forests (FFs) reduce the eutrophication of aquatic systems downstream by retaining or transforming phosphorus (P) received in floodwaters, but little is known about the pathways of P retention and/or transformation during flooding. The objective of this research was to identify specific mechanisms of P retention and transformation during flooding in FFs typical of the southeastern (SE) United States (US). To accomplish this objective, I added $^{32}\text{PO}_4^{3-}$ dissolved in river water to soil cores collected from topographic lows (swales) ($n = 12$ per site) at 2 Alluvial (AL) (Pee Dee; Wateree) and 2 Blackwater (BW) (Black River; Drowning Creek) FFs in the SE US. Cores were flooded with labeled river water and then incubated for 40 h or 8 d prior to sequential fractionation to extract P and associated aluminum (Al) and iron (Fe) cations in six soil P fractions. I hypothesized that $^{32}\text{PO}_4^{3-}$ would be transformed to organic form (P_o) in river and headwaters by physico-chemical complexation with organic matter (OM) via metal (Al and/or Fe) ligands (OM-metal-P), and/or by biological (microbial) uptake and conversion to P_o . Although I had expected transformation processes to occur within the water column in both AL and BW rivers, 77 % of added $^{32}\text{PO}_4^{3-}$ was transformed to P_o within 2 h of addition in BW river waters, while in AL river waters, all added $^{32}\text{PO}_4^{3-}$ remained in inorganic form. However, when labeled river waters were added to soil mesocosms, upon contact with the soil surface, additional transformation was observed resulting in an increase in the proportion of $^{32}\text{P}_o$ in headwaters, averaging 68 and 80 % of total ^{32}P recovered in AL and BW cores, respectively. Across all soil cores, average 12 and 0.5 % of the total ^{32}P added was recovered from head- and drain-waters, respectively. The remaining 87.5 % of added ^{32}P was recovered in soils, distributed within the soil fractions (73 %), microbial biomass (9 %) and roots (6 %). The proportion of $^{32}\text{P}_i$ and $^{32}\text{P}_o$ in cores (sum of P in the soil fractions + microbial biomass + roots + drainwaters) averaged 69 and 31 % in AL compared to 53 and 47 % of total ^{32}P recovered in BW cores. These results suggest that while physico-chemical processes dominate the retention of P in AL soils, P transformation and retention in BW systems are likely a combination of both biological and physico-chemical processes in BW soils. I also hypothesized that soil fractions would differ with respect to their P, Al, and Fe chemistries, and that the dominant mechanism of P retention in FF soils will be linked primarily to Al, clay minerals, and/or Fe sorption. The majority of ^{32}P recovered in soils was associated with Al/clay bound (NaOH-P), reductant-soluble (BD-P), residual (Res-P), and humic acid (HA-P) fractions. More Al than Fe was extracted in the NaOH

and Res fractions, and ^{32}P recovered in these fractions was positively correlated with total Al and soil clay content. Higher concentrations of Fe than Al were extracted in the BD- and Ca-bound (HCl-extractable) fractions. The ^{32}P recovered in these fractions was significantly positively correlated to Fe content, suggesting that in these acidic soils, P in these fractions is most likely bound to Fe. Although ^{32}P recoveries were dominated by the NaOH, BD, Res, and HA fractions, specific activity was highest in the water-soluble P fraction, root/rhizosphere and microbial pools, suggesting the importance of these biologically mediated (readily available P) pools in the short term retention of P during flooding events. These results confirm the importance of Al, clay content, and Fe in controlling P retention in FF soils, particularly in the SE US, and are consistent with previous reports of the importance of biological and physico-chemical in controlling short-term P retention and transformation in AL and BW FFs in the SE US.

3.1. INTRODUCTION

3.1.1 Floodplain Forests of the Southeastern US

Floodplain forests (FFs) are located in stream and river floodplains that are periodically inundated by overbank flooding (Brinson 1993, Mitsch and Gosselink 2007). In the southeast (SE) United States (US) (i.e., Virginia, North Carolina, South Carolina, and Georgia), FF ecosystems are associated primarily with two types of river systems: 1) alluvial (AL) rivers, whose waters arise in Piedmont or montane physiographic provinces; and 2) blackwater (BW) rivers, whose waters arise entirely within the Coastal Plain (Wharton and Brinson 1979, Hupp et al. 2005).

Blackwater rivers tend to be smaller than AL rivers, with lower gradient flows that have limited potential to entrain or erode sediments. Alluvial rivers have higher sediment loads and higher concentrations of inorganic ions, but lower concentrations of total organic carbon (TOC) compared to BW rivers; pH, hardness, and specific conductance also tend to be higher in AL than in BW rivers (Wharton and Brinson 1979, Hupp et al. 2005). Blackwater rivers have greater bacterial production rates and higher microbial biomass as compared to AL rivers (Edwards and Meyer 1986, Edwards et al. 1990, Meyer 1990, Leff and Meyer 1991, Entry 2000, Ochs et al. 2010).

Previous research in FFs of the SE US has suggested that complexation with organic matter via metal (Al, Fe) ligands (OM-metal-P) may be an important mechanism of inorganic phosphorus (P_i) retention and/or transformation in FFs (Lockaby and Walbridge 1998, Axt and Walbridge

1999, Darke and Walbridge 2000, Hogan et al. 2004). This mechanism could be particularly important in BW FFs, where sediment deposition is expected to play a minor role in P retention as compared to AL systems (Axt and Walbridge 1999, Darke and Walbridge 2000). Plant tissue, litterfall, and soil nitrogen (N):P ratios suggest that primary productivity in SE US FFs is generally N-limited (Lockaby and Walbridge 1998). Lower average litterfall N:P ratios in AL as compared to BW FFs, averaging 7.8 and 13.0 respectively (Lockaby and Walbridge 1998, Hupp et al. 2005), suggest that AL systems may be more strongly N-limited than BW systems, perhaps due to the higher sediment-associated P inputs received in floodwaters.

Floodplain forests are topographically heterogeneous environments in which microsite elevation has a direct influence on hydroperiod (Walbridge and Lockaby 1994, Stanturf and Schoenholtz 1998). In general, topographic lows (swales) have comparatively long hydroperiods and fine-textured, clayey soils typical of sediments underlying low energy waters (Hupp and Morris 1990, Craft and Casey 2000); topographic highs (ridges), as remnants of old levees and meander scrolls, have comparatively short hydroperiods and coarse, sandy soils typical of sediments underlying higher energy waters (Stoeckel and Miller-Goodman 2001, Hupp et al. 2005). In an Ogeechee River, GA FF, Darke and Walbridge (2000) found significant positive correlations between oxalate-extractable Al and soil OM, suggesting that the Al active in P_i retention could be in organic form (i.e., complexed to OM). Concentrations of OM-Al- PO_4^{3-} complexes were significantly greater in swale microsites that experience longer hydroperiods and have higher OM contents than higher elevation ridges. Because total P concentrations are consistently higher in swale microsites, swales may function as ‘hotspots’ of P retention and transformation (Darke and Walbridge 2000, Stoeckel and Miller-Goodman 2001, Wright et al. 2001).

3.1.2 Phosphorus Retention and Transformation

In both natural and constructed wetland soils and sediments, P retention capacity is controlled by both biological (plant uptake; microbial immobilization) and geochemical (P sorption) factors that affect the retention of both P_i and P_o forms (Richardson 1985, House et al. 1995, Nguyen et al. 1997). Suspended materials (particulate P_o and P_i adsorbed to mineral sediments) are removed from the water column during flooding by sedimentation, a physical process associated with a decline in the energy of floodwaters as they encounter increased resistance from the floodplain surface (Kleiss 1996, Hupp et al. 2005). Phosphorus retention in wetland soils is variously attributed to P sorption by Al and Fe minerals, OM-metal-P complexation, and/or interactions with clay minerals (Richardson 1985, Furumai et al. 1989, Paludan and Jensen 1995, Nguyen 2000, Hoffmann et al. 2009, Vu et al. 2010).

Soil Al and Fe minerals range from poorly ordered (amorphous or poorly crystalline) to highly structured (crystalline) forms. Because of their comparatively large surface area per unit soil volume, poorly crystalline (amorphous) forms of Al and Fe, rather than more crystalline forms, have been suggested to control the adsorption and precipitation of dissolved P_i in acid soils typical of FFs (Walbridge and Struthers 1993, Lockaby and Walbridge 1998). In addition to poorly crystalline mineral forms of Al and/or Fe, both metals form complexes with soil organic matter that can also sorb P_i (Axt 1997, Darke et al. 1997, Lockaby and Walbridge 1998, Axt and Walbridge 1999, Darke and Walbridge 2000). The formation and/or persistence of OM-metal-P complexes is strongly influenced by flooding regime (Peretyazhko and Sposito 2005), suggesting a potential role for soil organic matter and OM-metal-P complexation in controlling P sorption in

FFs. Many wetland studies have cited either total or oxalate-extractable Al as the best predictor of P sorption in wetland soils (Richardson 1985, Axt 1997, Darke and Walbridge 2000, Hogan et al. 2004). While the higher sediment loads carried by AL rivers and flood waters contribute to the greater observed rates of P retention in AL floodplains, the higher OM contents of BW river waters could promote P retention through the formation of OM-metal-P complexes.

Microorganisms can also retain (immobilize) a substantial amount of P_i , but this pool turns over rapidly, possibly due to the lysis of microbial cells during unfavorable environmental conditions (e.g., prolonged anaerobic conditions following a flooding event) (Richardson 1985, Walbridge and Vitousek 1987). Studies have shown that adding inorganic or low molecular weight organic P (P_o) to river waters can significantly increase bacterial abundance and production rates in both BW (Mallin et al. 2001, Mallin et al. 2004) and AL (Mohamed et al. 1998, Castillo et al. 2003) streams and rivers. Higher microbial biomass and bacterial production rates in BW (compared to AL) rivers could also control P_i retention and immobilization in these systems (Edwards et al. 1990, Basu and Pick 1997, Ensign and Mallin 2001, Ochs et al. 2010).

Phosphorus pools in river waters flowing into FFs are characteristically dominated by P_i forms, while the P in outflow waters is predominantly P_o (Elder 1985, Van Der Lee et al. 2004, Noe and Hupp 2007). Although some forms of P_o can also cause the eutrophication of downstream waters (Chu 1946, Turner and Newman 2005, Dunne et al. 2006), in general these observations suggest that the transformation of P_i to P_o could be an important process occurring during flooding events that improves water quality downstream. Dissolved P_i entering a FF via

overbank flooding could be transformed to P_o through biosynthetic pathways associated with plant and/or microbial uptake, or by OM-metal-P complexation (an abiotic process), prior to its export downstream (Cembella et al. 1984a, 1984b, Ridal and Moore 1993). The higher OM content, greater bacterial production rates, and higher microbial biomass of BW versus AL rivers could be partially responsible for the higher observed rates of P_i transformation in BW rivers (Edwards and Meyer 1986, Edwards et al. 1990, Meyer 1990, Leff and Meyer 1991, Entry 2000, Mallin et al. 2004, Ochs et al. 2010), but in both AL and BW systems, the potential importance of OM-metal-P complexation must also be considered.

3.1.3 Use of radioactive tracers and sequential fractionation schemes to assess P retention and transformation processes in wetland soils

Radioisotope studies have revealed important biological and geochemical processes in wetland P cycling, particularly with respect to P transformation processes in the water column of rivers, streams, and lakes, the retention and/or transformation of P in soils/sediments, and the soil/sediment–water interface of wetland ecosystems. Several studies have documented the importance of inorganic P (carrier-free $H_3^{32}PO_4$) uptake by microbes in the water column, and the subsequent transformation and release of P_o in rivers and streams (Newbold et al. 1983, Mulholland et al. 1985, Freeman and Lock 1995, Tate et al. 1995). The proportion of added $H_3^{32}PO_4$ converted to P_o form has been shown to correspond with increases in microbial biomass and/or TOC concentrations (as a proxy for OM content) in coastal river waters (Sabater et al. 1993, van Beusekom and Brockmann 1998, Pakulski et al. 2000, Flynn 2008). Transformation of inorganic to organic P in waters upon contact with surface soil/sediment has

been documented in rice paddies that were drained and flooded with $\text{Ca}(\text{H}_2\text{PO}_4)_2\text{-H}_2\text{O}$ (Sah and Mikkelsen 1986), and with $\text{H}_3^{32}\text{PO}_4$ added to surface sediment suspensions of a shallow lake (Istvanovics 1993), who noted that the transformation of inorganic to organic P was completely inhibited in sterilized (formalin treated) sediment.

While applying radioisotope tracers to lake, stream, river, or floodwaters has effectively quantified the microbial contribution to P transformation in the water column, assessing the biological vs. geochemical contribution to P retention and transformation in soils requires combining ^{32}P addition with a detailed sequential fractionation technique. Combining such schemes (e.g., Hedley et al. 1982, Psenner et al. 1984, Paludan and Jensen 1995) with the partitioning of inorganic and organic ^{32}P in soil pools (Jayachandran et al. 1992), allows discrimination and quantification of the amounts of P retained and/or transformed in specific soil fractions (Furumai et al. 1989, Di et al. 1997, Nguyen 2000). Phosphorus fractionation is routinely used to characterize soil P_i and P_o (see reviews by Cross and Schlesinger 1995, Levy and Schlesinger 1999, Reddy et al. 1999), but only a few fractionation methods distinguish between inorganic P associated with Al oxides and reducible Fe, as well as P bound to organic matter or humic acids, in wetland soils and sediments. One such scheme is a modified version of the Paludan and Jensen technique (Axt 1997, Hogan et al. 2004), that identifies P, Al, and Fe associated with six soil P fractions (**Figure 3.1**) -- water soluble P ($\text{H}_2\text{O-P}$), bicarbonate dithionite extractable P (BD-P), sodium hydroxide extractable P (NaOH-P), humic acid P (HA-P), hydrochloric acid extractable P (HCl-P), and residual P (Res-P). The $\text{H}_2\text{O-P}$ fraction estimates of loosely sorbed porewater P. The BD fraction extracts P adsorbed onto the surfaces of oxidized Fe and manganese. Sodium hydroxide-extractable P includes P sorbed onto the

surfaces of clays and Al oxides. The HA fraction estimates P associated with organic matter (i.e., humic acids), isolated via acidification of the previous NaOH-P fraction followed by filtration of the organic matter precipitate. Hydrochloric acid P primarily accounts for calcium-bound P in alkaline soils where Ca is a predominant cation, however, in acidic soils (typical of FFs) Ca-phosphates are unstable and P sorption to Fe and Al are favored (Freeman and Rowell 1981, Pierzynski et al. 1990). The Res-P fraction estimates both refractory organic and inorganic (i.e., occluded) P. While demonstrating the relative distribution of soil P into various inorganic and organic pools, fractionation schemes alone cannot provide information on the mechanisms of P retention and transformation. However, radioactive tracers (e.g., ^{32}P or ^{33}P) combined with P fractionation can identify the specific retention and/or transformation of added P into these soil fractions (Furumai et al. 1989, Di et al. 1997, Nguyen 2000).

Several studies have described rapid and substantial incorporation of ^{32}P or ^{33}P into wetland soil/sediment P pools (Richardson and Marshall 1986, Furumai et al. 1989, Walbridge 1991, Cooke 1992, Istvanovics 1993, Nguyen 2000, Kellogg and Bridgham 2003, Scinto and Reddy 2003, Vu et al. 2010) and mineral soil P fractions (Di et al. 1997, He and Zhu 1997, Daroub et al. 2000, Blake et al. 2002, Buehler et al. 2002, Asfary et al. 2004, Bunemann et al. 2004, Olander and Vitousek 2004). However, few studies have used a detailed sequential P fractionation scheme (like the Paludan and Jensen 1995 procedure) to track the fate of ^{32}P introduced to wetland soils. In a sewage impacted (Nguyen 2000) and coastal (Vu et al. 2010) wetland, total ^{32}P recovered by sequential P fraction (i.e., Olsen and Sommers (1982); Tiessen and Moir (1993), respectively) was highest in the NaOH and bicarbonate extractable fractions. Phosphorus sorption capacities in wetland soils have been found to be largely correlated with

amounts of Al and/or Fe extracted and soil clay fraction, or to a lesser extent soil Ca and OM content (Richardson 1985, Paludan and Jensen 1995, Hoffmann et al. 2009). The assumption is that the correlation between elements (Al, Fe, and Ca) and/or clay content in each fraction of a sequential extraction procedure, or in associated extracts (total Al, Fe, Ca, and OM, clay content), indicates that P is likely bound to these reactants. Although added P bound by geochemical reactants in the less-available (BD-P, NaOH-P, HA-P, and HCl-P) and occluded (Res-P) soils fractions may also be important for long-term P storage in soils, many studies have reported that readily available or biologically mediated (H₂O-P, root/rhizosphere, and/or microbial) pools are equally important, due to their higher ³²P compared to P content (specific activity), indicative of the sink strengths of these pools and their effectiveness as short-term stores for P (Richardson 1985, Walbridge 1991, Nguyen 2000, Kellogg and Bridgham 2003, Vu et al. 2010).

3.1.4 Objectives and Hypotheses

Although it is well established that FFs remove inorganic P from incident surface and subsurface waters, storing it within the wetland ecosystem (retention), and/or converting it to less biologically active forms (transformation), the specific pathways of P retention and transformation, and their relative contributions to improving water quality, have not been unequivocally demonstrated. The overall objective of this research was to elucidate specific mechanisms of P retention and transformation in FFs in the SE US. To accomplish this objective, I conducted an artificial flooding experiment in the laboratory with intact soil cores collected from four FFs (n = 2 per river type) in the SE US Coastal Plain, adding ³²P to river

waters prior to experimental flooding, and then using a detailed sequential P fractionation technique (Psenner et al. 1984, Paludan and Jensen 1995), identifying inorganic and organic ^{32}P in each soil pool (Jayachandran et al. 1992), to determine if AL and BW FFs retain and/or transform P during flooding events, if river/floodplain type affects these processes, and to identify the dominant mechanism(s) whereby inorganic P received in floodwaters is either retained in soil or transformed in the water column and/or at the sediment/soil interface, prior to export downstream. Because they are considered to be hotspots of P retention/transformation (Darke and Walbridge 2000, Stoeckel and Miller-Goodman 2001, Wright et al. 2001), I used soils collected from swale microsites only. Soil cores were flooded with water collected from the adjacent river at each site, that was subsequently labeled with carrier free $\text{H}_3^{32}\text{PO}_4$. I measured P and ^{32}P , Al, and Fe in each soil fraction, as determined by sequential fractionation (Paludan and Jensen 1995). I also estimated microbial biomass and root-/rhizosphere-associated P in soils. These data were used to test two hypotheses:

1) Mechanisms of P_i retention and transformation in FFs will be dominated by:

1a. biological processes – e.g., retention via biotic uptake and subsequent transformation of P_i to P_o .

1b. physico-chemical processes – e.g., complexation to OM via metal (Al, Fe) ligands and/or sorption to Al and/or Fe cations.

1c. some combination of biological and physico-chemical processes.

1d.

2) Processes of P_i retention and/or transformation in river waters and soils:

2a. would differ predictably between AL and BW FF systems

2b. would not differ between the two FF systems

3.2 METHODS

3.2.1 Site Description, Sample Collection and Preparation

Sites were selected in part, based on similarities in geological substrate (Dress et al., *in preparation*). The Pee Dee (PD) and Wateree (WT) sites are FFs found adjacent to AL river systems; the Black River (BL) and the Drowning Creek (DC) sites are floodplains adjacent to BW rivers. Although the probability of flooding in these sites is fairly predictable (~ 60 % of annual flooding events occur between January and April), flooding events can vary in intensity, duration, and number from year-to-year. Due to an unusually dry winter in 2006 and spring in 2007, however, no flooding events were recorded at any of these sites prior to or during soil collection. Location, drainage area, soil type, and dominant woody vegetation for each site are described in **Table 3.1**.

Soils samples were collected in May 2006. At each site, intact soil cores (n = 18 per site) were collected to a depth of 10 cm (excluding surface litter) in topographic lows (swales) using 5 cm diameter PVC cores. Cores were sealed at each end with rubber caps, and then stored on ice until returning to the laboratory, where they were stored at 4 °C. For each site, two randomly selected cores were used to estimate the volume of labeled solution that would be needed to saturate soils, and ensure standing water above the soil surface, and four cores were randomly selected for pre-flooding measurements of pH, moisture content, total C (TC), N, P, and soil P fractionation (methods described in detail below). Bulk density was estimated by measuring the

field-moist weight of each core and converting it to dry-mass equivalent per unit volume of soil based on percent moisture and core volume.

3.2.2 ^{32}P radioactive tracer experiment:

River water collected from each site was labeled with ^{32}P and then used to flood each soil core. These artificial flooding solutions were prepared by adding 185 MBq of carrier-free $\text{H}_3^{32}\text{PO}_4$ to 1.5 L of river water. Each core ($n = 8$ per site) was flooded with 150 mL of labeling solution, determined to completely flood cores as identified above, delivering approximately 7.0 MBq of ^{32}P per core. The actual activity added to each core was subsequently determined by counting 0.5 mL aliquots of each solution following labeling. Following flooding, cores were incubated in darkness at room temperature in plexi-glass boxes for 40 h or 8 d ($n = 4$ per incubation time) in the laboratory prior to soil P fractionation and ^{32}P analysis. Differences in the ^{32}P content of head-waters, drain-waters, and various soil P fractions were used to estimate the relative importance of P physico-chemical and biological retention and transformation processes occurring during flooding.

3.2.3 Soil sequential extraction and analysis:

At the end of each incubation period, 2 mL of head-water solution were removed for analysis from each core. Cores were then allowed to drain (by gravity or occasionally with vacuum suction applied), drainwaters collected, and the soil in each core was extruded from its PVC container. Following homogenization and the removal of large roots and rhizomes, duplicate 0.5

g dry weight equivalent (Pretty et al. 2003) subsamples of each soil sample were extracted using the sequential fractionation procedure of Paludan and Jensen (1995), as modified by Axt (1997) (**Figure 3.1**), and the P content and ^{32}P activity of each extract was estimated. Unless otherwise noted all extractions were performed in acid-washed 50 mL centrifuge tubes at 21 °C under continuous horizontal shaking. Water soluble P was extracted by shaking soil samples with 30 mL of deionized water for 1 hr. The residue from this step was extracted for 1 hr with 30 mL of 0.1 M $\text{Na}_2\text{S}_2\text{O}_4/\text{NaHCO}_3$, a strong reducing agent, to extract reductant-soluble (primarily Fe- and Mn-bound) P. Water soluble and bicarbonate/dithionate extracts received 1.2 and 5.6 mL of H_2SO_4 , respectively, to prevent microbial activity and the precipitation of desorbed P in reaction. In the third step of the sequential extraction procedure, P associated with clay minerals and oxides of Al was extracted with 30 mL of 0.1 M NaOH for 16 hr. Humic acids were precipitated from the NaOH supernatant by acidification to pH 1 with 1M H_2SO_4 , filtered, combusted at 500 °C in a muffle furnace overnight, and then boiled in 1M HCl in block digester for 1 hr. Residual P was recovered by oven-drying the remaining soil residue, grinding it with a mortar and pestle, and then combusting it at 550 °C for 14 hr in a muffle furnace. Approximately 0.2 g of this residue were extracted with 20 mL of 1 M HCl in a block digester for 1 hr. After each extraction, soil residues were centrifuged (15 min, 3400 rpm), filtered (Supor 0.45 μm filters), and the supernatants stored at 4 °C for further analysis. Also after each extraction, remaining soil pellets were rinsed by shaking with 30 mL of deionized water for 5 min, centrifuging for 10 min at 3400 rpm, and then, filtering (using the same filters used for the original extraction), and the resulting supernatants combined with the original supernatants. Bicarbonate/dithionate and NaOH fractions were washed once with their respective reagents, and then subsequently with deionized water as described above.

3.2.3 Microbial biomass P:

In addition to the six sequential fractions, P held in microbial biomass was estimated on separate, duplicate 0.5 g soil subsamples from each core by the chloroform-fumigation direct extraction technique (Brookes et al. 1982). One subsample was extracted with 30 mL of 0.5 M sodium bicarbonate (NaHCO_3) for 16 hrs to estimate available P, while a second subsample was fumigated with 0.5 mL chloroform (CHCl_3) overnight, and then extracted with NaHCO_3 as described above. Total P was determined for both samples colorimetrically after persulfate digestion of the solution (Qualls 1989), and microbial biomass P was calculated as the difference in P extracted from fumigated and non-fumigated sub-samples.

3.2.4 Analytical and chemical analysis:

Total ^{32}P radioactivity was measured on duplicate 0.5 mL aliquots of head- and drain-waters, and all soil extracts from both the sequential fractionation and microbial biomass P procedures, using a liquid scintillation counter (LS 6500 Liquid Scintillation Counter, Beckman Coulter, Fullerton, CA). Prior to counting, $^{32}\text{P}_i$ was separated from $^{32}\text{P}_o$ in head- and drain-waters, microbial-P extracts, and H_2O , BD, NaOH, and HCl soluble fractions, by the butanol separation method of Jayachandran et al. (1992). Aliquots (5 mL) of each extract were shaken with acidified ammonium molybdate dissolved in isobutanol. Inorganic ^{32}P complexed with ammonium molybdate is soluble in isobutanol; $^{32}\text{P}_o$ remains in the aqueous phase. Inorganic ^{32}P activity was measured in duplicate extractions (0.5 mL) from the isobutanol phase by liquid scintillation as described above. The proportion of $^{32}\text{P}_o$ was determined as the difference between total ^{32}P and MR- ^{32}P . The ^{32}P radioactivity (counts per minute, cpm) was measured by β counting using the

external standard method of quench correction. Total ^{32}P incorporated in roots and larger organic matter (separated from the bulk soil in each core) was measured by Cerenkov scintillation counting. Activities were adjusted for background and decay using the built-in ^{32}P half-life correction in the LS 6500 Liquid Scintillation Counter. Total P in labeled river-, head- and drain-waters, and in all soil P fractions, was determined after persulfate digestion of each extract (Qualls 1989). Digested extracts were then analyzed for inorganic P using the molybdate blue color reaction (Murphy and Riley 1962) on an Autoanalyzer 3 colorimeter (Method #G-175-96, Braun & Lubbe, Norderstedt, Germany). Organic P for these fractions was then estimated as the difference in molybdate reactive P (MRP) in digested vs. non-digested solution.

Total Al and Fe concentrations in soil extracts from the sequential fractionation procedure (both ^{32}P -labeled and non-labeled cores) were measured on a Varian Spectra 220FS atomic absorption spectrophotometer with a GTA-110 graphite tube atomizer (Varian Corp. Adelaide, Au). Soil pH was measured on fresh soils in a 1:2 slurry with deionized water. Total soil P was determined on duplicate sub-samples (0.2 g) from each ^{32}P -labeled and non-labeled core using a modified Kjeldahl digest technique (Parkinson and Allen 1975). Total P in the digest solutions was measured colorimetrically by the molybdate blue reaction (Method # G-189-97, Braun & Lubbe, Norderstedt, Germany). TOC and TN concentrations in unfiltered river water samples were measured on a TOC-V/TNM-1 analyzer (Shimadzu Scientific Instruments, Columbia, MD). Soil TC and TN concentrations were measured on dried soils from each core by dry combustion on a NC2500 elemental analyzer (ThermoQuest Italia S.P.A, Italy). Subsamples (10 g) of wet soil from each core were dried (110 °C) to a constant mass to determine soil moisture content. Soil texture was determined on one composite sample for each site (non-labeled cores)

by the Bouyoucos method (Bouyoucos 1962).

3.2.5 Statistical Analysis

Significant differences in ^{32}P activity (core^{-1}), P concentrations (g m^{-2}), specific activity ($\text{SA} = ^{32}\text{P}/\text{P}$), Al, Fe, N, C, and soil moisture at each site were determined by two-way ANOVA ($\alpha = 0.05$), with main effects of floodplain type (AL vs. BW) and incubation time (unlabelled, 0 h, and 8 d), with site nested within floodplain type, and the interaction terms. Data were log transformed where necessary to meet the assumption of normality. Relationships between soil properties, Al, Fe, and ^{32}P activity, P concentrations, and SA were determined by Pearson's correlation analysis with Bonferroni probabilities reported. All statistical analyses were performed using SYSTAT version 12 (SSI, San Jose, CA).

3.3 RESULTS

3.3.1 General characteristics of river water and ^{32}P addition

River water pH averaged 5.6, was highest in the WT (7.1) and lowest in DC (pH 5.0) (**Table 3.2**). Average pH in AL rivers was 7.0, and was significantly higher than pH in BW rivers (5.7). Total dissolved P concentrations ranged from 12.9 (DC river) to 61.2 (BL) $\mu\text{g L}^{-1}$. Average TDP, MRP, and P_o did not differ significantly between AL and BW rivers. Total N concentrations in river waters averaged $0.85 \pm 0.1 \text{ mg L}^{-1}$, and did not differ significantly between AL and BW rivers. However, river water TOC concentrations were significantly higher in BW compared to AL systems (**Table 3.2**).

Within 2 h of adding an average 190 μCi of carrier-free $\text{H}_3^{32}\text{PO}_4$ to each 1.5 L river water sample, 24 to 77 % of the $\text{H}_3^{32}\text{PO}_4$ added to BW river waters was converted to organic form (**Table 3.2**), but 100 % of the ^{32}P added to AL river waters remained in inorganic form. Both total ^{32}P and $\text{MR-}^{32}\text{P}$ concentrations were significantly higher in AL compared to BW river waters.

3.3.2 Head and drain water and soil P over 40 h and 8 d of incubation

Average total P contents and ^{32}P recoveries in head- and drain-waters or associated with the roots and microbial biomass did not differ significantly by floodplain type, site, or incubation time (**Table 3.3**). Averaged across all cores, TDP was significantly higher in drainwaters ($70.7 \pm 6.8 \mu\text{g L}^{-1}$) than in headwaters ($27.2 \pm 5.2 \mu\text{g L}^{-1}$). Total soil P estimate as the sum of P extracted in

the six soil fractions or by Kjeldahl digest was significantly higher at the WT, but similar in PD, BL, and DC soils (**Table 3.3 and 3.4**). Total soil P estimated by these two techniques did not differ significantly (paired t-test, $p = 0.496$).

Average total ^{32}P recoveries in soil fractions (sum of P extracted from the six soil fractions in the Paludan and Jensen scheme minus microbial biomass P) did not differ significantly by floodplain type, site, or incubation time (**Table 3.3**). Across all soil cores, only 12 and 0.5 % of the total ^{32}P added was recovered from head- and drain-waters, respectively. The remaining 87.5 % of added ^{32}P was recovered in soils, distributed within the soil fractions (72.7 %), microbial biomass (8.9 %) and roots (5.9 %). A majority of the ^{32}P recovered in headwaters was $^{32}\text{P}_o$, averaging 68 % vs. 80 % of total ^{32}P activity in the headwaters of AL and BW cores, respectively (**Table 3.7**). Whereas the proportion of $^{32}\text{P}_o$ vs. $^{32}\text{P}_i$ recovered in the remaining core (sum of P in the soil fractions + microbial biomass + roots + drainwaters), averaged 69 and 31 % in AL cores and 53 vs. 47 % in BW cores.

3.3.3 General chemistry and distribution of P in soil fractions:

3.3.3.1 General soil chemistry

Similar to river waters, pH was significantly higher in AL (5.1) than in BW (4.8) soils (**Table 3.4**). Soil pH in non-flooded cores was 4.7 and increased significantly in cores inundated with labeling solution, measuring 5.0 at both 40 h and 8 d after inundation. Total soil C and N concentrations averaged $2,469.6 \pm 146.1 \text{ g m}^{-2}$ and $217.8 \pm 23.9 \text{ g m}^{-2}$ respectively across all

sites, and were not significantly different as a function of floodplain type, site, or incubation time. Average clay content in AL soils (49.3 ± 2.8 %) was significantly higher (t-test, $P < 0.008$) than in BW soils (37.1 ± 3.3 %). Moisture content was highest in BL soils, followed by WT, PD and DC soils (**Table 3.4**).

3.3.3.2 P content and ^{32}P recovered in soil fractionation scheme

The majority of soil P was found in the NaOH-extractable fraction (47.2 ± 1.4 %), followed by Res (20.2 ± 1.9 %) $\approx$ BD- (18.7 ± 2.2 %) $>$ HA- (12.0 ± 0.6 %) $\gg$ HCl- (1.9 ± 0.1 %) $\approx$ H₂O-extractable (0.1 ± 0.0 %) fractions (**Tables 3.5**). Concentrations of H₂O-P, NaOH-P, HA-P, HCl-P, and Res-P differed significantly among the four sites, and similar to trends seen with total soil P, concentrations were highest at the WT site, followed by the BL and PD sites, and lowest at the DC site. Flooding of soil cores resulted in a significant decrease in BD-extractable P in cores inundated for 8 d. The P in the NaOH-extractable fraction was predominantly organic, P pools in the H₂O-extractable fraction was predominantly inorganic (**Tables 3.7**).

Similar to trends seen with the dominant soil P fractions, the majority of added ^{32}P was recovered in the NaOH-extractable, followed by the BD- $>$ Res $\approx$ HA- $\gg$ HCl- $\approx$ H₂O-extractable fractions (**Tables 3.6**). Average total ^{32}P recoveries in individual soil fractions did not differ significantly by floodplain type, site, or incubation time. The $^{32}\text{P}_o$ content (% of total ^{32}P) ranged from 2.9 to 28 % across all fractions; ^{32}P associated with the H₂O-, BD-, NaOH-, and HCl-extractable fractions was predominantly inorganic (**Tables 3.7**).

3.3.3.3 Specific Activity ($^{32}\text{P}/\text{P}$ ratio) in soils

Specific activities ($\text{SA} = ^{32}\text{P}/\text{P}$ ratio) were used to assess whether soil P pools (soil P fractions, microbial biomass P, and root P) had reached isotopic equilibrium (**Table 3.8**). Averaged across all soil cores, SAs were highest in the H_2O extractable fraction, followed by root biomass $\gg$ the microbial pool, followed by the $\text{BD} \approx \text{NaOH} \approx \text{HA} \approx \text{HCl} \approx \text{Res}$ soil fractions. While average SAs across all soil cores did not significantly differ in individual soil fractions/pools between 40 h and 8 d of flooding, statistically significant differences in SAs were observed within each site over the incubation period (ANOVA, site $\times$ incubation interaction, **Appendix Table 3.6, Figure 3.2**). The SAs in the H_2O and HCl extractable fractions in the PD soils, and the microbial biomass and Res-P fractions in the DC soils, decreased significantly over 8 d of flooding; similar decreases were also observed in $\text{H}_2\text{O}-\text{P}_i$ ($\text{H}_2\text{O}-\text{SA}_i$) and microbial P_o (microbial- SA_o) pools in both the PD and DC soils (**Appendix Table 3.6, Figure 3.2 a, d**).

3.3.4 Distribution of total Al and Fe in soil fractions

Soil Al content averaged $2,489.3 \pm 387.1 \text{ g m}^{-2}$, and was significantly higher (paired t-test, $P < 0.000$) than soil Fe content ($1,656.8 \pm 235.2 \text{ g m}^{-2}$), largely due to significantly higher Al compared to Fe concentrations in the Res, NaOH, and H_2O extractable fractions (paired t-test, $P < 0.000$) (**Table 3.9**). Fe extracted from the BD and HCl fractions was significantly larger than the Al content in these fractions (paired t-test, $P < 0.000$). Concentrations of Al and Fe extracted in the HA fraction were not statistically different (paired t-test, $P < 0.910$). Total Al (estimated as the sum of the six soil fractions) was significantly higher in WT, as compared to BL, PD, and DC soil (ANOVA, site effect: $F = 14.097$, $p = 0.012$). The majority (71.9 %) of Al was found in

the Res ($2247.9 \pm 377.0 \text{ g m}^{-2}$) fraction, followed by the NaOH (11.2 %) > HA (4.8 %) $\approx$ HCl (4.7 %) >> BD (4.5 %) >>> H₂O (2.9 %) extractable fractions (**Table 3.9**). Total Al and Al extracted from the HA, HCl, and Res fractions were not significantly different in non-flooded cores versus cores flooded for 40 h or 8 d. Average Al extracted from the NaOH, BD, and H₂O fractions was highest in non-flooded cores, but decreased significantly in flooded cores (**Table 3.9**).

The majority of soil Fe was found in the BD-extractable (43 % of total soil Fe) and Res (37.6 %) fractions, followed by the HCl- (13.1 %), HA- (3.3 %), NaOH- (1.9 %), and H₂O-extractable (0.9 %) fractions (**Table 3.9**). Fe extracted from soil fractions were generally higher in AL compared to BW soils. Average Fe extracted from the BD and HA fractions of AL floodplain soils was 951.3 ± 80.5 and $79.9 \pm 9.2 \text{ g m}^{-2}$, respectively; these Fe concentrations in AL soils were significantly higher than Fe concentrations in the same fractions of BW soils (203.5 ± 30.8 and $12.8 \pm 2.1 \text{ g m}^{-2}$, respectively). Iron concentrations in the H₂O and HCl fractions were highest in non-flooded cores, and decreased significantly in flooded cores (**Table 3.9**).

3.3.5 Correlations

Stepwise linear regression analysis was conducted on molar ratios of Fe:P/³²P vs. Al:P/³²P, Fe:P vs. Al:P, and Fe:³²P vs. Al:³²P to examine relationships between total Al or Fe and P content or ³²P recovered in each soil fraction. Results of these analyses are presented in **Table 3.10**. Both total P and ³²P in the H₂O-P fraction were strongly correlated with total Al extracted in the same fraction ($r^2 = 0.92$ and 0.86 , respectively, **Figure 3.3 a-b**). Total ³²P recovered was significantly

positively correlated with total Al extracted in the NaOH-P fraction ($r^2 = 0.53$, **Figure 3.3 c**), and the proportion of $^{32}\text{P}_i$ associated with this fraction was strongly correlated with soil clay content ($r^2 = 0.89$) (**Figure 3.3 d**). A significant correlation ($r^2 = 0.84$) also was observed between total ^{32}P recovered and total Al extracted from the Res-P fraction (**Figure 3.3 e**). Total ^{32}P recovered was significantly positively correlated with total Fe in the BD- and HCl-extractable fractions ($r^2 = 0.72$ and 0.66 , respectively, **Figure 3.3 f-g**). Total Al and Fe extracted and total ^{32}P extracted in the HA-P fraction was significantly positively correlated, and by removing a single outlier point (circled, **Figure 3.3 h**), the correlation between Fe^{32}P and Al^{32}P associated with this fraction decreased from a r^2 value of 0.82 to 0.38 (**Table 3.10**).

3.4. DISCUSSION

3.4.1 *General soil chemistry*

Total soil P, C, and N were within the ranges reported for forested wetland soils in the southern US (c.f. Lockaby and Walbridge 1998, Bedford et al. 1999) (**Table 3.3**). Lockaby and Walbridge (1998) used differences in N and P stoichiometric ratios in litterfall to estimate nutrient limitation in FF of the SE US, and suggested that N:P ratios >12 or <12 represent P and N limitation to productivity, respectively. Molar N:P ratios in soil cores ranged from 1.3 – 29.7, and were generally higher in BW (average N:P of 13) compared to AL FF (N:P = 3), suggesting that AL soils are likely to be more strongly N limited than BW soils (Lockaby and Walbridge 1998).

Total P estimated by sequential fractionation was predominantly associated with the NaOH-, Res, BD-, and HA-extractable fractions (**Table 3.5**). A similar rank order in dominant soil P fractions has been observed in other forested wetland (Axt and Walbridge 1999, D'Angelo 2005, Loeb et al. 2008), freshwater wetland (Ivanoff et al. 1998, Paludan and Morris 1999, Nguyen 2000, Qiu and McComb 2000, Hogan et al. 2004, Surridge et al. 2007), and coastal marsh soils (Paludan and Morris 1999, Morse et al. 2004), as well as lake sediments (Reitzel et al. 2006, Hu et al. 2007, Lake et al. 2007). Although total P content did not differ between non-flooded and flooded soils, a small but significant release of P from the BD-extractable fraction was observed in flooded cores. The release of P from this fraction may have contributed to the significant increase of total dissolved P observed in drainwaters compared to headwaters. The molar ratio of Fe:Al was 27 in the BD extractable fraction, and although total Fe did not differ significantly over the flooding period, flood induced reduction and dissolution of Fe (III) phosphates to

soluble Fe (Bossio et al. 2006) may have resulted in reduced P sorption capacity within this fraction (Kuo and Mikkelsen 1979, Paludan and Jensen 1995, Darke et al. 1997, Lockaby and Walbridge 1998, Darke and Walbridge 2000, Hogan et al. 2004, Quintero et al. 2007, Surridge et al. 2007, Loeb et al. 2008). Total Fe content in the H₂O and HCl extractable fractions, however, decreased significantly in flooded cores, and may have contributed to reduced P sorption capacity in these fractions. Decreases in Al extracted from the H₂O, BD, and NaOH fractions also were observed. Darke and Walbridge (2000) reported decreases in total Al with seasonal flooding in the Ogeechee FF soils, and suggested that this loss may have occurred due to the suspension of particulates and/or the solubilization of Al with flooding. In the current study, total Al and Fe (sum of all soil fractions) did not differ between non-flooded cores and cores flooded for 40 h and 8 d, and as such there were no net losses of Al and Fe from the soils (to drain- or headwaters) in mesocosm cores.

3.4.3 ³²P transformation in river-, head-, and drain-waters

Total dissolved P and MRP concentrations were similar in water samples collected from the AL and BW rivers, but within 2 hr of ³²P addition, up to 77 % of added ³²PO₄³⁻ was transformed to organic form in BW river waters. In AL river waters, all added ³²P remained in inorganic form. As predicted (in hypothesis 2a) the transformation of ³²PO₄³⁻ to ³²P_o occurred only in BW river waters where TOC concentrations were significantly higher than in AL river waters. The relatively low TOC content in AL compared to BW river waters, which potentially includes bacterial cells associated as particulate organic C in the water column, may have not been sufficient for detectable rates of P transformation in the 2 h post ³²P-label addition. As a proxy

for OM content, TOC in both AL and BW rivers was significantly correlated with the proportion of $^{32}\text{P}_o$ in river waters ($r^2 = 0.81$, $F = 66.313$, $P < 0.000$), suggesting that transformation of P in the water column may be due to microbial uptake of $^{32}\text{PO}_4^{3-}$ and subsequent release as $^{32}\text{P}_o$, and/or formation of OM-metal- ^{32}P complexes.

In the liquid-extraction method developed by Jayachandran et al. (1992), isobutanol and acidified molybdate used for partitioning inorganic from organic ^{32}P , free orthophosphate reacts with molybdenum forming phosphomolybdate which is extracted and quantified in the isobutanol phase, whereas $^{32}\text{P}_o$ remains in the aqueous phase. Inorganic ^{32}P activity in the isobutanol phase was measured by liquid scintillation, and the proportion of $^{32}\text{P}_o$ was determined as the difference between total ^{32}P and MR- ^{32}P . The use of acidified molybdate in this procedure may result in two potentially important sources of error. First, complex inorganic P forms such as pyrophosphate and polyphosphate (**Figure 2.2 b-c**) are not molybdate-reactive, and are therefore included in the organic P fraction (McKelvie et al. 1995, Turner et al. 2005). Second, a majority of P complexed with OM via metal (Al, Fe) ligands (OM-metal-P) is released by hydrolysis upon reaction with molybdate, and OM-metal-P is determined as free orthophosphate in solution (Bedrock et al. 1997, Gerke 2010). These errors are usually assumed to be negligible in freshwaters, although neither sources of error has been investigated systematically for river waters. While a majority of the $^{32}\text{P}_o$ detected using this method may have resulted from biological transformation and are considered “true” organic P compounds, I can only conclude that the mechanisms involved in the transformation of ^{32}P -label in BW rivers are a combination (as stated in hypothesis *1c*) of the biotic uptake of $^{32}\text{PO}_4^{3-}$ and subsequent release as $^{32}\text{P}_o$ (hypothesis *1a*) as well as the OM-metal- ^{32}P complexation (hypothesis *1b*).

Sabater et al. (1993) observed a high degree of correspondence between bacterial numbers and high/intermediate molecular weight DOC in a BW river (Ogeechee River, GA). The higher OM content, and larger microbial biomass and bacterial production rates documented in BW vs. AL rivers (Edwards and Meyer 1986, Leff and Meyer 1991, Basu and Pick 1997, Ensign and Mallin 2001, Castillo et al. 2004, Ochs et al. 2010) may have contributed to the significantly larger proportion of $^{32}\text{PO}_4^{3-}$ transformed to $^{32}\text{P}_o$ forms in BW compared to AL rivers. As much as 90 % of added $^{32}\text{PO}_4^{3-}$ has been shown to be removed from the water column, >50 % of which may be incorporated in microbial biomass (Newbold et al. 1983, Mulholland et al. 1985, Freeman and Lock 1995, Tate et al. 1995).

Upon flooding mesocosm cores, sustained inorganic to organic ^{32}P transformation was observed in ^{32}P -labeled river water (headwaters) used to flood cores. A majority of the ^{32}P in headwaters was converted to organic form, averaging 71 % of total ^{32}P activity after 40 h and 77 % after 8 d of incubation across all AL and BW cores. Similar to transformations observed within the water column in BW river water samples, upon contact with the soil surface nearly all added $^{32}\text{PO}_4^{3-}$ was transformed to $^{32}\text{P}_o$ in both AL (68 %) and BW (80 %) headwaters. Although a majority of the $^{32}\text{P}_o$ in BW headwaters resulted from transformations in river waters occurring within 2 h post labeling, river waters used to flood AL soil cores contained only inorganic ^{32}P , the subsequent transformation to $^{32}\text{P}_o$ may be attributed to increased biogeochemical reactivity on contact with the soil surface or due to microbial uptake. The proportion of $^{32}\text{P}_o$ increased significantly in headwaters as compared to river waters, upon contact with the soil surface,

averaging 18 % (DC cores), 41 % (Brandao et al. 2011), 58 % (WT), and by as much as 85 % (PD) in cores (two-sample t-test, $p < 0.000$). Microbial activity (estimated by the amount of total ^{32}P and the proportion of $^{32}\text{P}_\text{o}$ incorporated in microbial biomass, **Table 3.3 and 3.7**, respectively) and total soil C did not differ significantly between AL and BW soils, suggesting similar transformation rates in AL and BW systems. Rapid transformation of P_i on contact with surface soil/sediment has been documented in rice paddies that were drained and flooded with $\text{Ca}(\text{H}_2\text{PO}_4)_2\text{-H}_2\text{O}$ (Sah and Mikkelsen 1986), and with carrier-free $\text{H}_3^{32}\text{PO}_4$ added to surface sediment suspensions of a shallow lake (Istvanovics 1993). Istvanovics (1993) noted that $^{32}\text{P}_\text{i}$ transformation to $^{32}\text{P}_\text{o}$ was completely inhibited in sterilized (formalin-treated) sediment, highlighting the importance of biological P transformation. In **Chapter 2**, I observed higher NMR-visible total DIP concentrations in natural floodwaters entering the floodplain, along with the net increase in biogenic DOP forms in outflow waters. These results in addition to the transformation of $^{32}\text{P}_\text{i}$ to $^{32}\text{P}_\text{o}$ in headwaters (observed in the current study) upon contact with the soil surface are consistent with the theory that FFs regulate river water nutrient concentrations through the biotic transformation of nutrients during flooding events (Lockaby and Walbridge 1998, Hupp et al. 2005). Although ^{32}P transformation has been observed in other freshwater wetlands (Sah and Mikkelsen 1986, Istvanovics 1993), this study presents primary insight into the rapid and substantial transformation of inorganic to organic P in the water column, within the river waters in BW systems, and upon contact with the associated FF soils in AL systems. These findings underline the importance of these unique wetlands, their capacity to improve water quality downstream, as well as key differences in AL and BW system functions in the SE US.

Total ^{32}P recovered in headwaters averaged 12 % across all cores and was significantly lower in drainwaters (0.5 %), amounting to a 22 fold decrease over the 8 d incubation period. Similar to headwaters, a majority of the ^{32}P recovered in drainwaters was organic, averaging 82 % of total ^{32}P activity. This helps to clarify that $^{32}\text{PO}_4^{3-}$ transformed to $^{32}\text{P}_o$ is preferentially released to drainwaters, and that this occurs in both AL and BW soils. The proportion of $^{32}\text{P}_i$ and $^{32}\text{P}_o$ in cores (sum of P in the soil fractions + microbial biomass + roots + drainwaters) averaged 69 and 31 % in AL compared to 53 and 47 % of total ^{32}P recovered in BW cores. These results suggest that while physico-chemical processes (hypothesis *1b*) dominate the retention of P in AL soils, P transformation and retention in BW systems are likely a combination of both biological and physico-chemical processes (hypothesis *1c*) in BW soils.

3.4.4 Distribution and mechanism(s) of ^{32}P retention in soils:

Despite transformation of P_o , a substantial proportion (88 %) of ^{32}P added to artificial floodwaters was recovered in soil pools, distributed within the soil fractions (averaging 73 %), microbial biomass (9 %) and roots (6 %) across all AL and BW cores. A number of studies have described the rapid and substantial incorporation of ^{32}P or ^{33}P into wetland soil/sediment P pools (Richardson and Marshall 1986, Furumai et al. 1989, Walbridge 1991, Cooke 1992, Istvanovics 1993, Nguyen 2000, Kellogg and Bridgham 2003, Scinto and Reddy 2003, Vu et al. 2010) and mineral soil P fractions (Di et al. 1997, He and Zhu 1997, Daroub et al. 2000, Blake et al. 2002, Buehler et al. 2002, Asfary et al. 2004, Bunemann et al. 2004, Olander and Vitousek 2004). The proportion of microbial P recovered ranged from 2 to 15 % of total ^{32}P extracted from soils, and was comparable to estimates of P in depressional wetlands (4 - 6 %; Craft and Chiang 2002), fen

peatlands (12 - 13 %; Richardson and Marshall 1986), and other forested wetland soils (averaging 21 and 29 %; Wright et al. 2001, Hogan et al. 2004, respectively), but substantially less than ^{32}P immobilized in the microbial biomass in pocosin soils (averaging 90 %; Walbridge 1991). Surface roots are equally important in the removal of added ^{32}P from soil porewater, and although only 6 % of the added label was associated with the root biomass in the current study, which is comparable to the 2 – 4 % associated with the root and rhizosphere in the Everglades (Davis 1991), as much as 60 % of added ^{32}P was found to be associated with the root biomass in peatland soils (Jonasson and Chapin 1991).

My overall objective was to identify the major mechanisms of P retention and transformation in FFs. I found that a majority of added ^{32}P introduced in artificial floodwaters was recovered in the NaOH-, BD-, Res, and HA-extractable fractions. Of the few studies that have used a detailed sequential P fractionation scheme to track the fate of ^{32}P introduced to wetland soils, similarly high ^{32}P recoveries in the NaOH and bicarbonate extractable fractions were observed in sewage impacted (Nguyen 2000) and coastal (Vu et al. 2010) wetland soils. Yet to my knowledge, results presented in this chapter, represent the first attempt to identify dominant fates of ^{32}P and the mechanisms of ^{32}P retention and transformation in FFs during flooding. The ^{32}P -label delivered in headwaters was predominantly organic (averaging 74 % across all cores), and a significantly larger proportion of the ^{32}P recovered in drain waters was also organic (averaging 82 % across all cores), resulting from additional $^{32}\text{PO}_4^{3-}$ transformation to $^{32}\text{P}_o$ within the soil profile.

The majority of the total ^{32}P recovered in the NaOH-P and BD-P fractions was inorganic (**Table 3.7**). The substantial P retention capacity of wetland soils has been variously attributed to P sorption by soil Al and clay minerals, Fe, Ca, and OM content (Richardson 1985, Furumai et al. 1989, Paludan and Jensen 1995, Nguyen 2000, Hoffmann et al. 2009, Vu et al. 2010). The assumption is that the correlation of P sorption with Al and clay minerals, Fe, Ca, and OM, indicates that P is likely bound to these elements. Many wetland studies have cited Al (total Al, including oxalate-extractable Al) as the best predictor of P sorption (Richardson 1985, Axt 1997, Darke and Walbridge 2000, Hogan et al. 2004); soils in these studies had higher Al than Fe contents. More Al than Fe was extracted in the NaOH (Al:Fe = 12), Res (Al:Fe = 4), and HA fractions (Al:Fe = 2), however, a higher Fe content was associated with the BD extractable fraction (molar ratio of Fe:Al = 27), across all cores.

The BD and HCl extractable fractions comprised 43 and 13 %, respectively of the total Fe extracted in soil fractions across all AL and BW cores. Molar ratios of Fe: ^{32}P vs. Al: ^{32}P extracted from the BD and HCl fractions resulted in a significant positive correlation between total ^{32}P recovered and total Fe ($r^2 = 0.72$ and 0.66 , respectively, **Figure 3.3 f, g**) extracted, consistent with the generally higher Fe content associated with these fractions. Phosphorus associated with the HCl extractable fraction is primarily sorbed to Ca in alkaline soils, however, in acidic soils (typical of FF in the SE US) Ca-phosphates are unstable and P sorption to Fe and/or Al are favored (Freeman and Rowell 1981, Pierzynski et al. 1990). The predominance of Fe compared to Al content, and a strong positive correlation between Fe and P in these fractions has been reported in soils analyzed from riparian wetlands (Qiu and McComb 2000, Wang et al. 2006), freshwater wetlands (Nguyen 2000, Hansen et al. 2003), coastal lagoons (Lopez 2004),

salt marshes (Paludan and Jensen 1995, Paludan and Morris 1999, Coelho et al. 2004), and rice paddies (Sah and Mikkelsen 1986).

The largest proportion of ^{32}P recovered in soils was in the NaOH-extractable fraction, averaging 58 % of total ^{32}P recovered in all sequentially extracted soil fractions, and consistent with studies conducted on lake sediments (Furumai et al. 1989), wetland soils (Nguyen 2000, Vu et al. 2010). The dominance of P recovered by NaOH extraction was also reported for bottomland forested wetlands adjacent to the Tradewater river watershed in KY, where 90 % of added KH_2PO_4 in artificial floodwaters was recovered in this fraction (D'Angelo 2005). Soil P extracted using NaOH is primarily inorganic and considered bioavailable - loosely sorbed to Al and clay minerals. The proportion of $^{32}\text{P}_i$ associated with the NaOH-extractable fraction correlated best with clay content ($r^2 = 0.89$) across FF soils (**Figure 3.3 d**). Significant positive correlations between clay content and P sorption have been reported in floodplain soils along the Ogeechee River, GA (Darke and Walbridge 2000) and Danube River, FR (Lair et al. 2009). As expected, more Al than Fe was extracted from the Al/clay bound fraction, and total Al extracted was significantly correlated to total ^{32}P recovered in this fraction ($r^2 = 0.53$, **Figure 3.3 c**). Although the predominance of total Al (over Fe) in the NaOH fraction, and a significant positive correlation between Al and P or ^{32}P associated with this fractions has been reported in freshwater wetland (Paludan and Jensen 1995, Nguyen 2000), coastal swamps (Qiu and McComb 2000), lagoon (Lopez 2004), lake sediment (Reitzel et al. 2005). The current study is the first to document that similar mechanisms for the rapid (within 40 h of incubation) incorporation of NaOH-extractable P apply to FF wetland soils as well, in which (NaOH fraction) the largest

amount of ^{32}P introduced with flood waters was recovered (58 % of total ^{32}P extracted in soils fractions).

While a majority of the total ^{32}P in soils was retained in the NaOH-extractable fraction, the Res fraction accounted for 13 % of the total ^{32}P extracted from soils and had the largest proportion of total Al in all fractions, accounting for 72 % of total Al extracted by the Paludan and Jensen (1995) procedure. The predominance of Al relative to Fe in the Res-P fraction has been reported in other wetland soils (Axt and Walbridge 1999, Paludan and Morris 1999, Hogan et al. 2004). Molar ratios of $\text{Fe}:^{32}\text{P}$ vs. $\text{Al}:^{32}\text{P}$ in the Res fraction resulted in a significant correlation between total ^{32}P and Al ($r^2 = 0.84$, **Figure 3.3 e**), consistent with the higher Al content associated with this fraction. Since a majority of the Res-P pool represents refractory organic P, these results suggest that OM-metal-P complexation via Al (more than Fe) ligands may be an important mechanism of P_i retention within this soil fraction (Axt and Walbridge 1999, Darke and Walbridge 2000, Hogan et al. 2004). Similarly, higher Al than Fe was associated with the H_2O -extractable fraction ($\text{Al}:\text{Fe} = 3$), and total Al extracted was strongly correlated to both total P and ^{32}P recovered in this fraction ($r^2 = 0.92$ and 0.86 , respectively). As the most readily available and loosely sorbed fraction, a majority of P retained within the H_2O -P fraction would be complexed with OM via Al in FF soils (Axt and Walbridge 1999, Darke and Walbridge 2000, Hogan et al. 2004).

Although total Al and Fe measured were clearly defined by higher Al or higher Fe extracted from most soil fractions, the HA-P fraction was one exception, where more Al than Fe was

associated with the HA-P fraction in BW soils and higher concentrations Fe were extracted from this fraction in AL soil cores (**Figure 3.3 h**). These results clearly indicate that the Paludan and Jensen (1995) sequential P fractionation procedure is an effective method for sequentially extracting both sorbed P and the associated cations (Al and Fe) in each fraction, but is also able account for differences in sorption properties between different (AL vs. BW FF) soils within a fraction. Hogan et al. (2004) found similar shifts in the dominance of Al or Fe content in two forested wetlands (Braun vs. Barnstable sites) adjacent to the Chesapeake Bay, Kent Island, MD. In the current study, HA-Al was significantly correlated with total ^{32}P recovered in BW soils, whereas Fe extracted from the same fraction was correlated to total ^{32}P recovered in AL soils. BW floodplain soils generally contain higher levels of OM, and Al (compared to Fe) has a greater tendency to form OM-metal-P complexes (Paludan and Jensen 1995, Axt 1997, Hogan et al. 2004), which may be reflected in the predominance of Al associated with the humic acid fraction in BW soils.

3.4.5 Specific activity (ratio of $^{32}\text{P}/\text{P}$) in soil pools

After 8 d of flooding, the SAs of BD, NaOH, HA, HCl, and Res extractable fractions were similar and comparable to the average SA of the combined pools (soil fractions: indicated by a solid line and soil fractions + roots: indicated by a dashed line, **Figure 3.2**), suggesting that equilibrium with respect to P transfer between these fraction was being approached. After 40 h of flooding, the SAs of H_2O extractable fraction, microbial biomass and root/rhizosphere pools were comparatively larger than the average combined SAs of the soil fractions/pools (**Figure 3.2**). As the P content of these biologically mediated pools (H_2O -P, root and microbial P)

remained constant, the transfer of ^{32}P suggests P uptake among these fractions and pools. While significant decreases in SAs were observed between 40 h and 8 d of flooding in certain soil fractions/pools in PD and DC soils (**Figure 3.2 a, d**), when averaged across all sites, SAs of each soil pool/fraction did not differ significantly over 40 h and 8 d of incubation.

Although a majority of the ^{32}P added to soils was recovered in the NaOH, BD, Res, and HA extractable fractions, the highest SAs were observed in the H_2O extractable fraction, root/rhizosphere and microbial biomass pools (**Table 3.10**). These results clearly indicate that the biologically dominant and most available P pools (porewater, root/rhizosphere, and microbial) are important short-term sinks for P introduced in floodwaters. Overall, SAs in these pools did not statistically differ over incubation time, by site, or floodplain type, and as predicted in hypothesis 1a, these biologically mediated pools are effective at retaining P in both AL and BW FF soils over the entire period of flooding.

3.5 CONCLUSIONS

The dominant mechanism(s) of P retention in AL and BW FFs in the SE US Coastal Plain, or transformation in the water column and/or at the sediment/soil interface, was assessed in an artificial flooding experiment, with the addition of $^{32}\text{PO}_4^{3-}$ to river waters prior to flooding soils over a 8 d incubation period, and using a modified Paludan and Jensen (1995) P sequential fractionation procedure to assess the fate of P in soil fractions. A majority of the $^{32}\text{PO}_4^{3-}$ introduced to riverwaters was transformed to $^{32}\text{P}_\text{o}$ in BW river column, or in headwaters (AL and BW cores) upon contact with the soil surface. This rapid and substantial transformation of P suggests biotic uptake of $^{32}\text{PO}_4^{3-}$ and subsequent release as $^{32}\text{P}_\text{o}$ in floodwaters. Higher proportions of $^{32}\text{P}_\text{i}$ (69 % of total ^{32}P recovered) recovered in AL cores (sum of P in the soil fractions + microbial biomass + roots + drainwaters) indicate that physico-chemical processes dominate P retention in AL soils, but similar proportions of $^{32}\text{P}_\text{i}$ (53 %) and $^{32}\text{P}_\text{o}$ (47 %) in BW cores, suggests that both biological and physico-chemical processes contribute to P retention and transformation in BW soils. The majority of ^{32}P recovered in soils was associated with the NaOH-, BD-, Res, and HA-extractable fractions. Phosphorus introduced with floodwaters was either sorbed to Al and/or clay minerals in the NaOH-, Res, and H_2O -extractable fractions, or strongly associated with Fe in the BD- and HCl-extractable fractions in both AL and BW soils. The ^{32}P extracted from the HA fraction, however, differed between AL and BW soils; HA-Al was significantly correlated with HA-extractable ^{32}P in BW soils; HA-Fe was significantly correlated with HA-extractable ^{32}P in AL soils. Average total ^{32}P recoveries in the root/rhizosphere and microbial pool and sequentially extracted soil fractions did not differ significantly by floodplain type, site, or incubation time, which suggests that AL and BW FF soils are equally effective at retaining and transforming P introduced with floodwaters. Although

a majority of the ^{32}P added to soils was recovered in the NaOH, BD, Res, and HA extractable fractions, the highest SAs were observed in biologically dominant and most available P pools (H_2O extractable fraction, root/rhizosphere and microbial biomass pools), indicative of the sink strengths of these pool and their effectiveness at removing and storing P from floodwaters, thus reducing P inputs to aquatic systems downstream of these FFs in the SE US Coastal Plain.

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Figure 3.1: Sequential fractionation method used to extract P and ^{32}P from floodplain forest soils (Paludan and Jensen 1995).

Figure 3.2: Specific activity ($SA: ^{32}P/P$; $SA_i: ^{32}P_i/P_i$; $SA_o: ^{32}P_o/P_o$) of soil pools/fractions in flooded (for 40 h and 8 d) soil cores collected from the **a.** Pee Dee R., **b.** Wateree R., **c.** Black R., **d.** Drowning Crk. sites, and **e.** average of all cores across FF sites. Average combined SA of Paludan and Jensen sequentially extracted fractions (indicated by a solid line) and sequentially extracted fractions + roots (indicated by dotted line) indicative of isotopic equilibrium (IE) within exchangeable soil pools/fractions. * Indicates significant differences ($p \leq 0.05$) in SA between the 40 h and 8d incubation period (for ANOVA results see **Appendix Table 3.5**).

Figure 3.3: Comparison of the molar ratio of Fe:P vs Al:P in H₂O (**a – b**), NaOH (**c**), Res (**e**), BD (**f**), HCl (**g**), HA (**h**) extractable fractions and NaOH-³²P_i vs soil clay content associated with the Al/clay vs. soil clay content (**d**) in cores collected from AL and BW FFs. Solid line represents a ratio = 1 between variables.

Table 3.1: Locations and characteristics of the study sites. Soils data were obtained from regional soil maps (USDA/NRCS); drainage areas are from the USGS web interface for the National Water Information System (<http://waterdata.usgs.gov>)

River	Site	Latitude	Longitude	Drainage area (km ²)	Soil Class	†Dominant Vegetation
AL	PD	34° 12' N	79° 32' W	22,870	Aquic Quartzipsamments	Cypress, Tupelo
	WT	33° 49' N	80° 37' W	14,478	Fluvaquentic Dystrochrepts	Cypress, River Birch, Willow Oak
BW	BL	34° 45' N	78° 18' W	1,751	Typic Fluvaquent	Sweetgum, Tupelo
	DC	35° 03' N	79° 29' W	474	Fluvaquentic Dystrochrepts/Typic Fluvaquents	River Birch, Sweetgum

†The dominant woody vegetation at all four sites is mixed deciduous bottomland forest dominated by bald cypress (*Taxodium distichum*), sweetgum (*Liquidambar styracifula*), river birch (*Betula nigra*), water tupelo (*Nyssa aquatica*), and willow oak (*Quercus phellos*) (Dress et al. *in preparation*).

Table 3.2: Total P and ^{32}P , inorganic (MR-P) and organic P and ^{32}P , and TOC, TN, and pH in river waters collected at each AL and BW floodplain forest. * Indicates significant differences ($P \leq 0.05$) in measured components between alluvial and blackwater rivers using a two-sample t-test analysis.

River water chemistry	P concentrations			³² P activity			Chemical parameter		
Site and FF type	TDP (µg l ⁻²)	MRP (µg l ⁻²)	P _o (µg l ⁻²)	Total ³² P (µCi core ⁻¹)	MR- ³² P (µCi core ⁻¹)	³² P _o (µCi core ⁻¹)	TOC (mg l ⁻²)	TN (mg l ⁻²)	pH
PD	44.3	21.9	22.4	190.9	208.8	0.0	4.3	1.0	6.9
WT	51.3	32.6	18.7	201.2	206.7	0.0	4.3	0.9	7.1
<i>AL FF – mean</i>	<i>47.8 (3.5)</i>	<i>27.3 (5.4)</i>	<i>20.6 (1.9)</i>	<i>196.0 (1.9)*</i>	<i>207.7 (0.4)*</i>	<i>0.0</i>	<i>4.3 (0.0)</i>	<i>0.9 (0.0)</i>	<i>7.0*</i>
BL	61.2	37.0	24.2	186.0	141.4	44.7	12.3	1.1	6.4
DC	12.9	3.9	9.0	177.8	40.3	137.5	9.2	0.4	5.0
<i>BW FF – mean</i>	<i>37.1 (24.2)</i>	<i>20.5 (16.6)</i>	<i>16.6 (7.6)</i>	<i>181.9 (1.6)</i>	<i>90.8 (19.1)</i>	<i>91.1 (17.5)</i>	<i>10.8 (0.6)*</i>	<i>0.8 (0.1)</i>	<i>5.7</i>

Table 3.3: Mean ($\pm$ SE) total P content and ^{32}P activity recovered in headwater, soils, and drainwater in AL and BW cores, non-flooded, and cores flooded for 40 h and 8 d. Values shown in bold with different superscript letters correspond to means that are significantly different (ANOVA, $P < 0.05$ following Tukey's HSD test). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 3.1**. * Indicates significant differences ($p \leq 0.05$) in measured components between head- and drain-water samples using a paired t-test analysis.

Total P and ³²P	Head-water		Drain-water		Root		Microbial biomass		Soil fractions	
Site and FF type	P	³²P	P	³²P	P	³²P	P	³²P	P	³²P
	(µg l ⁻²)	(% Recov.)	(µg l ⁻²)	(% Recov.)	(g m ⁻²)	(% Recov.)	(g m ⁻²)	(% Recov.)	(g m ⁻²)	(% Recov.)
PD	24.3 (3.3)	17.4 (5.4)	69.8 (14.8)	0.1 (0.0)	0.3 (0.1)	6.7 (1.9)	0.6 (0.1)	6.0 (1.9)	34.2 (5.1)^B	69.7 (5.6)
WT	60.5 (14.8)	3.8 (1.4)	83.5 (6.6)	0.7 (0.3)	0.3 (0.1)	3.1 (0.9)	0.7 (0.2)	1.9 (0.7)	74.7 (3.5)^A	90.5 (1.7)
AL – mean	42.4 (8.7)	10.6 (3.2)	78.2 (6.9)	0.4 (0.2)	0.3 (0.0)	4.9 (1.1)	0.6 (0.1)	4.0 (1.1)	54.5 (5.5)	80.1 (4.0)
BL	18.5 (4.4)	7.8 (1.7)	81.7 (19.4)	0.6 (0.3)	0.4 (0.1)	7.8 (1.9)	0.8 (0.1)	12.3 (4.2)	38.0 (2.3)^B	71.5 (4.8)
DC	5.3 (0.7)	18.9 (2.2)	47.4 (7.2)	0.6 (0.2)	0.1 (0.0)	6.1 (2.1)	0.7 (0.0)	15.5 (3.9)	18.9 (2.2)^B	59.0 (4.9)
BW – mean	11.9 (2.7)	13.4 (2.0)	64.6 (10.9)	0.6 (0.2)	0.2 (0.1)	6.9 (1.4)	(0.8) (0.1)	13.9 (2.8)	28.5 (2.7)	65.2 (3.7)
Overall - mean	27.2 (5.2)	12.0 (1.9)*	70.7 (6.8)*	0.5 (0.1)	0.3 (0.0)	5.9 (0.9)	0.7 (0.1)	8.9 (1.7)	41.5 (3.7)	72.7 (3.0)
Incubation										
Non-flooded	---	---	---	---	---	---	0.9 (0.2)	---	53.4 (6.0)	---
40 h	22.3 (3.9)	10.7 (1.8)	74.4 (8.6)	0.5 (0.2)	0.3 (0.1)	5.9 (1.3)	0.7 (0.1)	11.1 (2.3)	39.8 (5.9)	71.9 (3.6)
8 d	32.0 (9.8)	13.3 (3.3)	66.1 (11.1)	0.5 (0.2)	0.2 (0.0)	6.0 (1.3)	0.6 (0.1)	6.8 (2.5)	37.1 (6.2)	73.5 (4.8)

Table 3.4: Mean ($\pm$ SE) total P content (analyzed by Kjeldahl digest) and general soil physical and chemical characteristics in control cores (unlabeled), and cores inundated for 40 h and 8 d across all AL and BW cores. Values shown in bold with different superscript letters correspond to means that are significantly different (ANOVA, $P < 0.05$ following Tukey's HSD test). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 3.2**.

	Soil chemistry					
Site and FF type	Total P (g m ⁻²)	C (g m ⁻²)	N (g m ⁻²)	pH	Moisture (%)	Clay (%)
PD	30.8 (3.3) ^B	1,833.9 (309.0)	112.7 (18.2)	5.1	27.7 (1.1) ^B	37.0
WT	92.9 (5.1) ^A	2,593.5 (226.0)	189.0 (16.8)	5.2	35.1 (2.2) ^{A B}	61.5
<i>AL FF – mean</i>	<i>61.8 (7.7)</i>	<i>2,213.7 (205.7)</i>	<i>150.8 (14.9)</i>	5.1 ^A	<i>31.4 (1.5)</i>	<i>49.3 (2.8)</i>
BL	34.5 (1.4) ^B	3,357.4 (212.8)	242.2 (17.3)	4.7	43.4 (1.9) ^A	51.5
DC	14.0 (1.5) ^B	2,093.4 (167.8)	233.3 (10.6)	4.8	27.6 (1.6) ^B	22.6
<i>BW FF – mean</i>	<i>24.3 (2.6)</i>	<i>2,725.4 (196.0)</i>	<i>237.7 (9.9)</i>	4.8 ^B	<i>35.5 (2.2)</i>	<i>37.1 (3.3)</i>
Incubation						
Non-flooded	44.4 (9.6)	3,046.5 (268.7)	213.8 (19.6)	4.8 ^A	31.0 (1.6)	---
40 h	45.8 (9.3)	2,505.5 (242.2)	211.1 (18.7)	5.0 ^B	32.1 (2.1)	---
8 d	39.6 (7.3)	2,145.2 (212.2)	167.7 (17.4)	5.0 ^B	36.0 (2.5)	---

Table 3.5: Mean ($\pm$ SE) of total P content in sequentially extracted fractions (Paludan and Jensen 1995) within control cores (not flooded) and soils flooded for 40 h and 8 d across all AL and BW cores. Values shown in bold with different upper case superscripts correspond to means that are significantly different (ANOVA, $P < 0.05$ following Tukey's HSD test). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 3.3**. Overall means represents the relative proportion (%) of total P in soil fractions across all AL and BW cores, where values shown with different lower case superscripts correspond to means that are significantly different (ANOVA, $P < 0.05$ following Duncan's multiple range test).

P (g m⁻²)	Distribution in soil fractions					
Site and FF type	H₂O-P	BD-P	NaOH-P	HA-P	HCl-P	Res-P
PD	0.0 (0.0) ^B	5.2 (0.6) ^{BC}	17.7 (3.3) ^{AB}	4.1 (0.7) ^{AB}	0.5 (0.1) ^B	7.3 (1.5) ^B
WT	0.1 (0.0) ^A	6.2 (0.4) ^{AB}	32.2 (1.7) ^A	7.6 (0.5) ^A	1.7 (0.1) ^A	27.5 (3.0) ^A
<i>AL FF – mean</i>	<i>0.1 (0.0)</i>	<i>5.7 (0.4)</i>	<i>24.9 (2.5)</i>	<i>5.9 (0.6)</i>	<i>1.1 (0.2)</i>	<i>17.4 (2.8)</i>
BL	0.0 (0.0) ^B	3.7 (0.2) ^C	22.1 (1.7) ^{AB}	5.6 (0.4) ^{AB}	0.8 (0.2) ^{AB}	6.5 (0.5) ^B
DC	0.0 (0.0) ^B	7.6 (0.6) ^A	7.9 (1.1) ^B	2.1 (0.3) ^B	0.4 (0.1) ^B	1.6 (0.2) ^C
<i>BW FF – mean</i>	<i>0.0 (0.0)</i>	<i>5.7 (0.5)</i>	<i>15.0 (1.9)</i>	<i>3.9 (0.5)</i>	<i>0.6 (0.1)</i>	<i>4.1 (0.6)</i>
<i>Overall – mean (% Prop.)</i>	<i>0.1 (0.0)</i> ^d	<i>18.7 (2.2)</i> ^b	<i>47.2 (1.4)</i> ^a	<i>12.0 (0.6)</i> ^c	<i>1.9 (0.1)</i> ^d	<i>20.2 (1.9)</i> ^b
Incubation						
Non-flooded	0.1 (0.0)	6.5 (0.8) ^A	28.8 (3.4)	6.0 (0.7)	1.3 (0.1)	11.6 (2.9)
40 h	0.0 (0.0)	6.3 (0.5) ^A	18.0 (2.3)	4.9 (0.6)	0.7 (0.1)	10.6 (3.2)
8 d	0.0 (0.0)	4.6 (0.4) ^B	17.5 (2.8)	4.3 (0.7)	0.7 (0.2)	10.5 (3.0)

Table 3.6: Mean ($\pm$ SE) ^{32}P recovered in sequentially extracted fractions (Paludan and Jensen 1995) within control cores (not flooded) and soils flooded for 40 h and 8 d across all AL and BW cores. Results showing significant effects and interaction terms of the 2-way ANOVA are listed in **Appendix Table 3.4**. Values shown with different lower case superscripts correspond to means that are significantly different (ANOVA, $P < 0.05$ following Duncan's multiple range test).

³² P (% recovery)	Distribution in soil fractions					
Site and FF type	H ₂ O- ³² P	BD- ³² P	NaOH- ³² P	HA- ³² P	HCl- ³² P	Res- ³² P
PD	1.5 (0.2)	16.1 (2.4)	60.4 (2.2)	10.8 (0.4)	1.2 (0.2)	10.0 (0.8)
WT	0.9 (0.1)	18.9 (1.1)	54.9 (1.6)	10.4 (0.4)	2.9 (0.3)	11.9 (1.3)
<i>AL FF – mean</i>	<i>1.2 (0.1)</i>	<i>17.5 (1.3)</i>	<i>57.6 (1.5)</i>	<i>10.6 (0.3)</i>	<i>2.1 (0.3)</i>	<i>11.0 (0.8)</i>
BL	0.8 (0.1)	9.3 (0.5)	61.0 (1.0)	11.9 (0.2)	2.4 (0.4)	14.7 (1.2)
DC	3.4 (0.9)	20.4 (1.3)	55.2 (1.1)	9.7 (0.7)	1.0 (0.1)	10.3 (1.3)
<i>BW FF – mean</i>	<i>2.1 (0.5)</i>	<i>14.8 (1.6)</i>	<i>58.1 (1.0)</i>	<i>10.8 (0.5)</i>	<i>1.7 (0.3)</i>	<i>12.5 (1.0)</i>
<i>Overall – mean</i>	<i>1.7 (0.3)^d</i>	<i>16.2 (1.0)^b</i>	<i>57.9 (0.9)^a</i>	<i>10.7 (0.3)^c</i>	<i>1.9 (0.2)^d</i>	<i>11.7 (0.6)^c</i>
Incubation						
40 h	1.6 (0.2)	15.8 (1.2)	57.3 (1.0)	10.5 (0.3)	2.1 (0.3)	12.7 (0.6)
8 d	1.8 (0.5)	16.6 (1.7)	58.5 (1.5)	10.9 (0.4)	1.6 (0.2)	10.7 (1.1)

Table 3.7: Mean ($\pm$ SE) of the proportion of organic P in TP measured in headwater, drainwater, microbial biomass, and sequentially extracted soil fractions within control cores (not flooded) and soils flooded for 40 h and 8 d across all AL and BW cores. Values shown in bold with different superscript letters correspond to means that are significantly different (ANOVA, $P < 0.05$ following Tukey's HSD test). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 3.5**. †Soil sum represents the sum of $^{32}\text{P}_o$ extracted from the six soil fractions in the Paludan and Jensen scheme + microbial biomass + root associated + drainwater.

P_o (% Prop. of TP)	Headwater	Drainwater	Microbial biomass		H₂O-P		BD-P	NaOH-P		HCl-P	†Soil sum
Site and FF type	³²P_o	³²P_o	P_o	³²P_o	P_o	³²P_o	³²P_o	P_o	³²P_o	³²P_o	³²P_o
PD	85.0	86.4	18.1	46.6	6.1	16.8	21.5	52.1	28.2	0.0	38.2
WT	50.8	81.1	59.0	42.9	11.5	4.8	2.4	32.0	7.8	0.0	24.6
<i>AL FF- mean</i>	<i>67.9</i>	<i>83.6</i>	<i>38.5</i>	<i>44.7</i>	<i>8.8</i>	<i>10.8</i>	<i>11.9</i>	<i>42.1</i>	<i>18.0</i>	<i>0.0</i>	<i>31.4</i>
BL	65.3	78.5	49.7	48.4	4.1	26.4	29.8	68.5	9.8	2.8	32.9
DC	95.4	81.2	18.0	29.7	1.2	39.2	58.1	72.8	64.7	8.9	61.2
<i>BW FF- mean</i>	<i>80.4</i>	<i>79.9</i>	<i>33.9</i>	<i>39.0</i>	<i>2.6</i>	<i>32.8</i>	<i>43.0</i>	<i>70.7</i>	<i>37.2</i>	<i>5.9</i>	<i>47.1</i>
<i>Overall- mean</i>	<i>74.1</i>	<i>81.7</i>	<i>36.2</i>	<i>41.9</i>	<i>5.7</i>	<i>21.8</i>	<i>27.0</i>	<i>56.4</i>	<i>27.6</i>	<i>2.9</i>	<i>39.2</i>
Incubation											
Non-flooded	---	---	---	---	6.0	---	---	59.3	---	---	
40 h	71.2	76.3	45.8	48.2	7.5	14.1	24.2	57.9	27.5	4.7	37.8
8 d	77.0	87.3	26.6	35.5	3.8	29.5	30.0	53.3	27.7	1.1	40.6

Table 3.8: Specific Activity ($SA: {}^{32}P/P$, $SA_i: {}^{32}P_i/P_i$, $SA_o: {}^{32}P_o/P_o$) in sequentially extracted fractions (Paludan and Jensen 1995) in soils flooded for 40 h and 8 d across all AL and BW cores. Results showing significant effects and interaction terms of the 2-way ANOVA are listed in **Appendix Table 3.5**. Values shown with different lower case superscripts correspond to means that are significantly different (ANOVA, $P < 0.05$ following Duncan's multiple range test).

SA	Soil pools and fractions											
	Root	Microbial			H ₂ O		BD	NaOH		HA	HCl	Res
AL FF	SA	SA	SA _i	SA _o	SA	SA _i	SA	SA	SA _i	SA	SA	SA
PD	11,091.9	6,699.8	3,697.4	25,900.4	45,364.6	37,905.8	1,123.5	2,038.9	3,125.1	1,270.1	2,211.0	1,154.1
WT	11,502.4	2,204.0	8,285.9	3,139.3	9,796.1	8,634.7	2,656.5	1,352.4	1,658.2	1,091.1	1,239.2	398.5
BW FF												
BL	11,640.6	7,263.1	6,548.5	13,200.1	30,445.4	11,605.5	1,331.6	1,673.0	5,000.4	1,221.0	2,048.7	1,525.6
DC	29,541.2	13,448.0	8,955.0	45,416.3	83,908.1	21,704.3	1,233.4	3,761.1	4,786.9	2,824.0	1,456.0	3,430.2
Overall –	<i>16,087.3</i>	<i>7,403.7</i>	<i>6,871.7</i>	<i>21,914.0</i>	<i>42,378.5</i>	<i>19,962.6</i>	<i>1,586.2</i>	<i>2,206.4</i>	<i>3,642.7</i>	<i>1,601.5</i>	<i>1,738.7</i>	<i>1,627.1</i>
Incubation												
40 h	15,167.6	10,169.1	9,250.8	37,540.2	59,868.5	32,117.0	1,459.9	2,379.2	3,894.8	1,520.2	2,138.6	2,049.2
8 d	16,949.5	4,638.4	4,492.4	6,287.8	24,888.6	7,808.2	1,712.6	2,033.5	3,390.5	1,662.8	1,338.8	1,205.0

Table 3.9: Mean ($\pm$ SE) distribution of total Al and Fe in sequentially extracted fractions (Paludan and Jensen 1995) within control cores (not flooded) and soils flooded for 40 h and 8 d across all AL and BW cores. Values shown in bold with different superscript letters correspond to means that are significantly different (ANOVA, $P < 0.05$ following Tukey's HSD test). For complete results showing significant effects and interaction terms of the 2-way ANOVA see **Appendix Table 3.8**. Values shown with different lower case superscripts (* for proportion of total Al) correspond to means that are significantly different (ANOVA, $P < 0.05$ following Duncan's multiple range test).

Al and Fe (g m ⁻²)			Distribution of Al and Fe in soil fractions									
	H ₂ O		BD		NaOH		HA		HCl		Res	
Site and FF type	Al	Fe	Al	Fe	Al	Fe	Al	Fe	Al	Fe	Al	Fe
PD	13.6	6.3	24.2	889.4	125.1	7.5	63.5	67.5	55.4	182.3	1,901.2	939.3
WT	12.7	3.9	21.9	1,013.2	100.3	11.6	63.8	92.2	83.8	490.9	5,550.5	1,840.5
<i>AL FF – mean</i>	<i>13.1</i>	<i>5.1</i>	<i>23.0</i>	<i>951.3^A</i>	<i>112.7</i>	<i>9.6</i>	<i>63.6</i>	<i>79.9</i>	<i>69.6</i>	<i>336.6</i>	<i>3,725.8</i>	<i>1,389.9</i>
BL	11.0	3.2	17.1	250.0	133.8	18.2	34.9	17.9	60.4	157.3	1,433.6	403.4
DC	15.4	4.6	23.7	157.0	62.3	7.9	26.6	7.8	16.1	17.6	106.5	37.8
<i>BW FF – mean</i>	<i>13.2</i>	<i>3.9</i>	<i>20.4</i>	<i>203.5</i>	<i>98.0</i>	<i>13.0</i>	<i>30.7</i>	<i>12.8</i>	<i>38.2</i>	<i>87.4</i>	<i>770.1</i>	<i>220.6</i>
<i>Overall – mean (% Prop.)</i>	<i>2.9</i>	<i>0.9</i>	<i>4.5</i>	<i>43.2</i>	<i>11.2</i>	<i>1.9</i>	<i>4.8</i>	<i>3.3</i>	<i>4.7</i>	<i>13.1</i>	<i>71.9</i>	<i>37.6</i>
Incubation												
Non-flooded	17.9	5.4	28.0	546.9	188.5	14.5	73.3	52.2	63.0	412.8	2,827.4	528.8
40 h	12.5	4.7	21.2	548.8	65.0	10.7	41.5	47.0	52.5	159.4	1,880.1	739.1
8 d	11.4	3.9	19.1	621.2	104.2	10.4	39.8	42.8	50.7	164.3	2,326.1	1,009.6

Table 3.10: Relationships between soil properties, Al, Fe, and ^{32}P activity, P concentrations, and SA were determined by Pearson's correlation analysis with Bonferroni probabilities reported. Data are log transformed where necessary.

		H ₂ O	BD	NaOH	HA	HCl	Res	Sum of fractions
Fe:P vs. Al:P	r ²	0.92	0.39	---	0.09	0.36	0.37	0.51
	F	420.818	25.858	n.s.	4.758	22.546	23.403	41.795
	P	≤ 0.0001	≤ 0.0001	---	0.035	≤ 0.0001	≤ 0.0001	≤ 0.0001
Fe:³²P vs. Al:³²P	r ²	0.86	0.72	0.53	0.38	0.66	0.84	0.81
	F	189.125	80.113	35.644	19.404	61.648	166.779	136.823
	P	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001
Fe:P/³²P vs. Al:P/³²P	r ²	0.95	0.72	0.47	0.55	0.70	0.80	0.81
	F	544.286	81.844	28.977	39.064	73.762	123.156	134.504
	P	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001

CHAPTER 4

Microbial Diversity and Function in Floodplain Forests of the Southeastern US

ABSTRACT

Floodplain forests (FFs) play an important role in improving water quality in downstream aquatic ecosystems through the retention and transformation of phosphorus (P) received via both upland hydrologic discharge and overbank flooding. Through a variety of processes (sediment deposition, soil adsorption, plant uptake, and microbial immobilization), FFs remove phosphates from the water column, storing them within the wetland ecosystem or converting them to less biologically active organic forms. While microorganisms are known to represent important short-term sinks for phosphate in freshwater wetland ecosystems, little is known about how microbial community composition affects P retention and transformation. This chapter focuses on understanding the physical and soil chemical factors (floodplain type and microtopographic relief within the floodplain) that control variations in bacterial and fungal community composition in FFs, and how these variations might influence the retention and transformation of P received in floodwaters. My specific objectives are to: 1) *investigate in the detail soil microbial community composition across microtopographic gradients in two FFs*; and 2) *compare changes in microbial community composition as a function of floodplain type and microtopographic elevation in eight FFs*. To address these objectives, I carried out a geographically extensive sampling of both alluvial (AL) and blackwater (BW) floodplain sites in the southeastern US; within these sites, I examined how variations in microtopographic elevation (ridge (RD) vs. swale (SW)) and soil nutrient status affect microbial community composition. The microbial community was assessed by combining bacterial-16S-rDNA and fungal-18S/ITS-rDNA cloning with denaturing gradient gel electrophoresis (DGGE) fingerprinting to screen clone libraries and identify dominant group members. Cluster analysis of DGGE profiles using Jaccards coefficient and UPGMA dendrograms, divided soils into two clusters that corresponded with differences in floodplain type (AL vs. BW floodplain soils). Detrended correspondence analysis (DCA) and cluster analysis of DGGE profiles were in close agreement and clearly separated the bacterial and fungal community composition of AL floodplain soils from BW floodplain soils. The bacterial community detected in the clone-library consisted of *Alpha*-, *Beta*-, *Gamma*-, *Delta*-proteobacteria, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Chloroflexi*, *Nitrospirae*, and *Bacteroidetes* bacterial groups; fungal taxa belonged to *Ascomycota*, *Basidiomycota*, *Zygomycota*, and *Chytridiomycota* subdivisions. In both the clone

library and DGGE fingerprint data, the dominant bacterial taxa belonged to the *Acidobacteria* and *Alphaproteobacteria* groups, and the most prevalent fungal taxa belonged to the *Ascomycota* and *Basidiomycota* subdivisions. Two-way ANOVA of soil chemical properties indicated that total P (TP) concentrations were significantly higher in AL compared to BW soils, whereas total C (TC), N:P, and C:P ratios were significantly higher in BW compared to AL floodplain soils. The DCA analyses of DGGE fingerprint data and the subsequent fitting of environmental variables (soil moisture, pH, TC, TP, total nitrogen, and microbial biomass P) to ordination plots, revealed that TP and pH were associated with the composition of bacterial and fungal communities in AL soils, whereas, TC was strongly correlated with the microbial community profiles in BW FFs. The combined results of these two studies provide taxonomic identification of the dominant bacteria and fungi in these floodplain soils, and show that differences in floodplain type (AL vs. BW) are associated with differences in soil TC, TP and pH, that are important predictors of microbial composition. Results should be broadly applicable to FFs in the southeastern US.

4.1. INTRODUCTION

Anthropogenic inputs of phosphorus (P) from agricultural activities and land use change have led to dramatic shifts in global P cycling, with considerable impacts on water quality and downstream aquatic ecosystems (Carpenter 2008). Because P is the most common limiting nutrient in freshwater ecosystems (Schindler 1978), increased P loading in runoff from agricultural and urban areas can lead to eutrophication of freshwater ecosystems and its associated effects -- degraded water quality, toxic algal blooms, anoxia, and loss of aquatic biodiversity (Carpenter 2005). Riparian wetlands, including floodplain forest (FF) ecosystems, physically and biogeochemically linking adjacent uplands and streams, and can therefore play a key role in ameliorating anthropogenic nutrient (i.e., P) loadings from both upstream and upslope sources (Lockaby and Walbridge 1998, Hupp et al. 2005). Floodplain forests (FFs) remove P from incident surface and subsurface waters, storing it within the wetland ecosystem (retention), and/or converting it to less biologically active form (transformation) (Walbridge and Struthers 1993, Hupp et al. 2005, Noe and Hupp 2009). Although it is well established that P retention and transformation processes are a function of both the abiotic sorption potential of soil aluminum (Al), iron (Fe), clay minerals and biotic uptake, microbes also strongly influence P immobilization in wetlands. A substantial proportion of total P (TP) in FF soils (21-29 %) may be stored in microbial biomass (Wright et al. 2001, Hogan et al. 2004), while as much as 75-90 % of TP in fen peatlands and pocosins may be in microbial form (Richardson and Marshall 1986, Walbridge 1991, Bedford et al. 1999). Despite the significant role of microorganisms in soil P dynamics, little is known about the metabolic processes or taxonomic composition of the bacteria and fungi involved in the retention and transformation of P in wetlands, especially FFs.

4.1.1 Phosphorus retention and transformation in FFs of the southeastern US

Wetlands cover 570 million ha (or 6 %) of the Earth's surface, approximately 15 % of which are FFs (Thorsell et al. 1997). An estimated 11 million ha of FFs remain in the US, a number that has declined by 50 % since the late 1970s, primarily due to land use changes (Mitsch and Gosselink 2007). In the southeastern (SE) US, FF ecosystems are associated primarily with two types of river systems: 1) Alluvial (AL) rivers, whose waters arise in Piedmont or montane physiographic provinces; and 2) Blackwater (BW) rivers, whose waters arise entirely in the Coastal Plain (Wharton and Brinson 1979, Hupp et al. 2005). Blackwater rivers tend to be smaller than AL systems, with lower gradient flows that have limited potential to entrain or erode sediments (i.e., Lockaby and Walbridge 1998). Alluvial rivers have higher sediment loads and higher concentrations of inorganic ions (including P), but lower concentrations of total organic carbon (C) compared to BW rivers; pH, hardness, and specific conductance also tend to be higher in AL than in BW rivers (Wharton and Brinson 1979; Hupp et al. 2005).

Both AL and BW FFs in SE US experience frequent flooding events (see **Chapter 2**) that can occur at any time during the year, but approximately 60 % of annual flooding events occur between January and April (Dress et al. *in preparation*). A number of studies have found that P pools in river waters flowing on to a floodplain are often dominated by inorganic P forms, while P pools in outflow floodwaters are dominated by organic P forms (Elder 1985, Craft and Casey 2000, Darke and Walbridge 2000, Stoeckel and Miller-Goodman 2001, Bruland and Richardson 2004, Van Der Lee et al. 2004, Bruland and Richardson 2005). Because they require extensive

enzymatic hydrolysis before they can be utilized by aquatic microorganisms, high molecular weight organic P forms carried with floodwaters that exit these wetlands generally are less bioavailable than DIP forms, and are thus less likely to cause the eutrophication of downstream aquatic ecosystems (Pant et al. 2002, McDowell and Koopmans 2006). Therefore, FFs are frequently thought of both as sinks for inorganic P and sources of organic P forms (Richardson et al. 1988, Brinson 1993, Lockaby and Walbridge 1998, Darke and Walbridge 2000, Bruland and Richardson 2004, Hupp et al. 2005). Floodplain forests improve water quality by retaining phosphates received in floodwaters within the wetland, and/or transforming them to organic form and then exporting them from the wetland ecosystem via hydrologic pathways (Richardson 1989, Lockaby and Walbridge 1998, Mitsch and Gosselink 2007). While it has been well-established that P retention in wetland soils is attributed to abiotic processes of sedimentation, sorption by Al, Fe, organic matter – Al/Fe - P complexation, and clay mineral interactions in soil fractions (Richardson 1985, Furumai et al. 1989, Paludan and Jensen 1995, Nguyen 2000, Hoffmann et al. 2009, Vu et al. 2010), biotic processes (i.e., plant and microbial P immobilization), and particularly the composition of FF soil microbial communities, have been relatively understudied. To date, a detailed assessment of the linkages between the taxonomic identity of bacterial and/or fungi, and the biogeochemical cycling of P in southeastern US FFs has not been done.

4.1.2 Microbial Community Composition and Ecosystem Function

Bacteria and fungi play a critical role in the global cycling of P (Pierrou 1976, Ruttenberg 2005, Filippelli 2008, Liu et al. 2008), which is coupled with the cycling of C, N, S, and oxygen in

both aquatic and terrestrial ecosystems (Kump and Mackenzie 1996, VanCappellen and Ingall 1996, Lenton and Watson 2000). Although recent reviews have synthesized the importance of linking taxonomic diversity (of bacterial and/or fungi) and biogeochemical function with respect to C, N and S cycling in soils/sediments (Jetten 2008, Muyzer and Stams 2008, Barton and Fauque 2009, Conrad 2009, Murrell and Jetten 2009, Canfield et al. 2010, McCarren et al. 2010), the specific link between functional diversity and P cycling is commonly overlooked (Gutknecht et al. 2006, Falkowski et al. 2008). This is especially true in wetlands, with previous work largely limited to estimations of biomass nutrient content (microbially incorporated C, N, and P) or the culturable microbes involved in C, N, S, and Fe cycling (Gutknecht et al. 2006, Hartman et al. 2008). To understand how changes in C, N, and P availability impact ecosystems requires comprehensive knowledge of how bacterial and/or fungal communities respond to changes in both nutrient concentrations and their stoichiometric ratios in soils (Grimm et al. 2003, Zak et al. 2006, Dinsdale et al. 2008).

Since FFs are depositional systems, the differences in the characteristics between AL and BW river waters could influence the amount and type of nutrients entering the floodplain. Blackwater rivers generally carry lower sediment loads compared to AL rivers, but have higher concentrations of dissolved organic carbon (DOC) (Wharton and Brinson 1979, Hupp et al. 2005). The amount, source, and quality of DOC can affect bacterial numbers, respiration rates, and overall microbial production (Findlay et al. 2003, Wiegner and Seitzinger 2004, Bossio et al. 2006, Jasser et al. 2009, Kobayashi et al. 2009, Adame et al. 2012). Soil C:N:P ratios can also affect key microbial processes, including rates of decomposition, denitrification, sulfate

reduction, P solubilization, and nutrient uptake in general (Golterman 1995, Kuehn et al. 2000, Sterner and Elser 2002, Mander et al. 2003, Zak et al. 2006, Corstanje et al. 2007, Yang et al. 2007, Burgin et al. 2011, Sorrell et al. 2011). In a depressional wetland in southwestern Georgia, Craft and Chiang (2002) reported a critical C:N ratio of 20:1 to indicate N limitation for microbial processes. Lockaby and Walbridge (1998) used differences in N and P stoichiometric ratios in litterfall to estimate nutrient limitation, suggesting that in the southeastern US, BW FFs (N:P > 12) tend to be more P limited than AL FFs (N:P < 12) FFs. The higher sediment associated P inputs carried by AL as opposed to BW floodwaters are thought to be are thought to be at least partly responsible for the lower N:P ratios observed in AL FFs. Differences in soil C:P ratios may also affect rates of microbial P retention and transformation. Gao and Heath (2005) found that DOC content in lake sediments can influence P uptake and cellular P concentrations. When sediment DOC concentrations are low, P was taken up at much higher rates and stored in the cell as polyphosphates (poly-P), creating a higher P quota for cells, whereas higher sediment DOC content resulted in reduced P uptake and lower % P incorporation in bacterial cells. Similarly, differences in soil C and P content in AL vs. BW FF soils could induce microbial population shifts associated with different efficiencies in P retention and transformation. Tezuka (1990) inoculated bacteria from lake sediments with a range of substrates varying in C/P concentrations, and reported that P immobilization rates were high (~60 % of total P in media) when substrate C:P ratios were < 40, but decreased abruptly with increasing substrate C/P concentrations until P uptake rates reached zero when C:P ratios were > 60. Microorganisms incorporate phosphate into cellular constituents and both bacteria and fungi are capable of luxury P consumption, where poly-P is stored in specialized vacuoles, significantly influencing P concentrations in the substrate or water column (Davelaar 1993, Khoshmanesh et

al. 2002, Hupfer et al. 2007, Szabo et al. 2011, Diaz et al. 2012). As much as 91 % of total P retention in a woodland stream (Elwood et al. 1981), and 75-90 % of total P in fen peatlands and pocosins (Richardson and Marshall 1986, Walbridge 1991) has been attributed to microbial uptake. A considerable amount of interest in identifying and characterizing poly-P accumulating microorganisms (PAO), is due partly for their ability to remove DIP from effluents in sewage treatment plants, commonly termed enhanced biological phosphorus removal (EBPR) systems (Serafim et al. 2002, Oehmen et al. 2007, Hupfer et al. 2008, Hirota et al. 2010). Taxa belonging to major bacterial subdivisions (*Acidobacteria*, *Alpha-*, *Beta-*, *Gammaproteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Nitrospirae*) efficient at P removal in EBPR systems also have also been detected in terrestrial, aquatic, and wetland ecosystems (Blackall et al. 2002, Hirota et al. 2010, Gebremariam et al. 2011).

Rates of P retention and transformation by soil microorganisms can vary spatially within a FF ecosystem (Wright 1998; Stoeckel and Miller-Goodman 2001), as well as temporally with seasonal changes in temperature and moisture availability (Wright et al. 2001). Floodplain forests are topographically heterogeneous environments in which microsite elevation has a direct influence on hydroperiod (Walbridge and Lockaby 1994, Stanturf and Schoenholtz 1998). Seasonal flooding appears to increase P availability in low-lying swale microsites by both physical transport of materials and decreased biological nutrient demand under prolonged anaerobic conditions (Mitsch and Gosselink 2000; Stoeckel and Miller-Goodman 2001). In general, topographic lows (swales) have comparatively long hydroperiods and fine-textured, clayey soils typical of sediments underlying low energy waters (Hupp and Morris 1990, Craft

and Casey 2000); topographic highs (ridges), such as remnants of old levees and meander scrolls, have comparatively short hydroperiods and coarse, sandy soils typical of sediments underlying higher energy waters (Stoeckel and Miller-Goodman 2001, Hupp et al. 2005). Because total P concentrations are consistently higher in swale microsites, swales may function as ‘hotspots’ of P retention and transformation (Stoeckel and Miller-Goodman 2001, Wright et al. 2001).

4.1.3 Current understanding of the microbial community composition in FF soils

Most attempts to assess the microbial contribution to biogeochemical function in floodplain soils have been limited to estimates of microbial biomass (Wainright et al. 1992, Ellis et al. 1998, Gessner et al. 1998, Simonovic and Simonovicova 1999, Baldwin and Mitchell 2000, Robinson et al. 2000, Stoeckel and Miller-Goodman 2001, Mubyana et al. 2003, Kutuzova et al. 2009, Teixeira et al. 2011, Palijan 2012), enrichment techniques such as BIOLOG-ecoplates (Kamitani et al. 2006), or culture based characterizations of specific bacterial species (Leff and Meyer 1991). While these methods provide estimates of microbial productivity, they do not provide information on the taxonomic and physiological distinctions of bacteria and/or fungi within complex soil communities (Rosado et al. 1997, Anderson and Cairney 2004, Nocker et al. 2007). Culture-independent methods like 16S rRNA terminal restriction fragment length polymorphism (Besemer et al. 2005, Song et al. 2008) or phospholipid fatty acid profiles (Rinklebe and Langer 2006, Miletto et al. 2008, Langer and Rinklebe 2009, Frohne et al. 2012), while providing estimates of bacterial vs. fungal contribution to soil community structure, are only capable of resolving microbial groups to within subdivision-level taxonomic identity (Muyzer 1999, Zelles 1999, Anderson and Cairney 2004, Simon and Daniel 2011). Taxonomic surveys of the complete bacterial and/or fungal community structure using molecular techniques are

comparatively rare in FF soils, and are limited to using specific PCR-primers to target only for sulfate-reducing (Acha et al. 2005, Miletto et al. 2008), methanogenic (Kemnitz et al. 2004), methanotrophic (Ross et al. 1997), or mercury-reducing (Oregaard and Sorensen 2007) prokaryotes. However, Lowell et al. (2009) utilized universal 16S-rDNA PCR-primers and denaturing gradient gel electrophoresis (DGGE) to target eubacterial populations in a small-scale floodplain adjacent to the Flathead River in Northwestern Montana. Sequence analysis of DGGE bands in this study, detected several operational taxonomic units (OTUs) belonging to the *Alpha*-, *Beta*-proteobacteria, *Acidobacteria*, *Actinobacteria*, and *Planctomycete* bacterial subdivisions. To date, a similar detailed assessment of fungal community structure in FF soils has not been attempted. More importantly, assessments that include soil chemical properties, and/or their stoichiometries, and their relationships to bacterial and/or fungal community structure and/or P retention and transformation processes in FF soils, have not been conducted.

4.1.4 Objectives and Hypotheses

The overall objective of this study was to examine variations in bacterial and/or fungal community structure as a function of floodplain type (AL vs. BW), microsite elevation (RD vs. SW), and associated variations in soil chemical parameters (total C, N, P, microbial P, soil pH and moisture content) in FFs of the southeastern US, and how these variations, in turn, might affect the retention and transformation of P. I used a molecular approach to identify dominant organisms in floodplain soils that combined bacterial 16S-rDNA and fungal 18S/ITS-rDNA cloning and DGGE fingerprinting. First proposed by Burr et al. (2006), this combined protocol takes advantage of the ability of cloning to package DNA and provide full-length sequence information for assessing the taxonomic identity of organisms, and of DGGE to display the

community profile of all organisms present, while screening for the presence–absence of dominant organisms in soil samples. I accomplished this objective, through two separate studies that used a combination of biogeochemical and molecular microbial techniques.

Study 1 - Topographic variation in bacterial and fungal community structure in an AL and a BW FF:

A detailed characterization of the bacterial and fungal community composition in ridge (RD, less-frequently flooded topographic highs, n = 6 composite ridge soils per floodplain type) and swale (SW, more-frequently flooded topographic lows, n = 6 composite swale soils per floodplain type) soils in one AL and one BW floodplain forest.

Study 2 – A comparative analysis of bacterial and fungal community composition across eight FFs:

Assess changes in bacterial and fungal community composition as a function of floodplain type (AL vs. BW) and microsite elevation (RD vs. SW) in eight FFs (n = 4 per floodplain type), sampling sites in VA, NC, SC, and GA, that represented a range of soil chemical and physical parameters in AL vs. BW FFs, to capture a range of conditions likely to affect bacterial and fungal community composition and diversity.

To my knowledge, this research is the first attempt at linking microbial community composition and ecosystem function with respect to P retention and transformation processes across several FFs in the southeastern US. A molecular microbial approach that combines bacterial 16S-rDNA and fungal 18S/ITS-rDNA cloning and DGGE fingerprinting to identify dominant group

members has broad potential applicability in terrestrial and wetland ecosystem ecology. Soil chemical (pH, % moisture, C, N, P, and microbial biomass P) analysis conducted on subsamples of soil in which the microbial community profiles were assessed provided a spatially extensive survey within and across floodplain types, maximizing variation in these factors in order to examine their effects on microbial community composition. I used a multivariate statistical approach (Oksanen et al. 2011) to compare changes in bacterial and fungal community structure with variations in soil chemical properties. *Both studies addressed the overall hypothesis that distributions of bacterial and fungal taxonomic groups in FFs can be predicted based on variations in the physical environment (i.e., floodplain type, microsite elevation) and soil chemical parameters (pH, % moisture, C, N, P, and microbial biomass P), and that resulting variations in microbial community composition affect processes of P retention and transformation in southeastern US FFs.* Based on a general understanding of the characteristics of AL and BW rivers and their associated FFs, I also tested the following specific hypotheses: 1) *that bacterial species diversity would consistently exceed fungal species diversity in all soils;* 2) *that bacterial and/or fungal community composition would vary predictably as a function of floodplain type (AL vs. BW) and microsite elevation (RD vs. SW) in FFs of the southeastern US;* 3) *that differences in C:N:P stoichiometry, pH and moisture content in AL vs. BW floodplain soils would co-vary with differences in bacterial and/or fungal community structure, revealing dominant microorganisms known for diverse metabolic activities related to C and N cycling and P immobilization;* and 4) *that bacterial and/or fungal community structure would vary spatially with changes in microsite elevation, with more frequently flooded SW soils that experience prolonged periods of anaerobic conditions revealing dominant organisms that immobilize P.*

A primary focus of this research is to contribute to the basic understanding of the potential controls over bacterial and fungal community structure, the microbial contribution to P retention and transformation, and overall metabolic function in FF soils. Particularly with respect to P cycling, microbial metabolic processes in wetlands are poorly understood and in need of synthesis (Gutknecht et al. 2006, Zak et al. 2006, Falkowski et al. 2008, Hartman et al. 2008, Oksanen et al. 2011). In order to associate metabolic function and relevant processes in wetlands to the taxonomic identity of microbes detected in FF soils, I conducted a broad literature comparison (*phylum*-, *class*-, *order*-, *family*-specific) of bacterial and fungal taxa with known biogeochemical functions in terrestrial, aquatic, and wetland ecosystems. Such comparisons may benefit our understanding of biogeochemical processes and microbial ecology across all ecosystems (Ruttenberg 2005, Zak et al. 2006, Filippelli 2008, Liu et al. 2008).

4.2 METHODS

4.2.1 Site Descriptions, Experimental Design, Sample Collection and Preparation

Two studies were conducted to understand the soil chemical factors associated with variations in bacterial and fungal community composition in FFs (i.e., floodplain type and microsite elevation), and how these variations, in turn, might be related to the retention and transformation of P received in floodwaters. A total of eight sites (**Table 4.1**) used in these two studies were established within 5 km of USGS gaging stations that continuously monitor river height. The Nottaway River (NT), Sebrell, VA; the Ocmulgee River (OC), Lumber City, GA; Pee Dee River (PD), Pee Dee, SC; and the Wateree River (WT), Eastover, SC, are FFs adjacent to AL rivers. The Black River (BL), Tomahawk, NC; Drowning Creek (DC), Hoffman, NC; the Salkehatchie River (SK), Miley, SC; and the Satilla River (ST), Waycross, GA, are FFs adjacent to BW rivers. Drainage area, soil type, and dominant woody vegetation at each site are described in **Table 4.1**. Study sites, experimental design, and sampling schemes for two studies were as follows:

Study 1 - Topographic variation in bacterial (TB) and fungal (TF) communities: To investigate soil microbial community composition across microtopographic gradients and variations in flooded conditions, rectangular plots 3 (BL) and 12 ha (WT) in size were established at one AL (WT) and one BW (BL) FF. Each plot was further divided into three 20 × 50 m subplots. Within each subplot, three surface (0 – 10 cm) soil samples were collected at each of four randomly chosen locations, and composited by location to produce 12 discrete soil samples per site, representing either low lying swale microsites, generally closer to the river bank, or less frequently flooded ridge microsites. All plots were established in summer 2002 and soils were collected in summer 2003.

Study 2 - Multi-site variation in bacterial (MB) and fungal (MF) communities: To compare changes in microbial community composition as a function of floodplain type, microsite elevation, and variations in flooded conditions. Similar to Study 1, rectangular plots (0.3 to 12 ha depending on site size) were established in each of four AL (NT, OC, PD, and WT) and four BW (BL, DC, SK, ST) sites. Each plot was divided into three 20 × 50 m subplots, and three surface soils samples (top 5 cm) were collected at four randomly chosen locations in each subplot, and then composited by location to produce 12 discrete soil samples per site. Plots were established in 2002 and 2003; soils were collected in summer 2003. To reduce the number of DGGE gel analyses, two composite soils from each site, representing one SW and one RD location, were chosen randomly for microbial community analysis.

All soils were collected using sterile 50 mL centrifuge tubes, sealed in sterile plastic bags, and stored on ice until returning to the lab where they were homogenized and extracted for genomic DNA within 24 hrs. Soil samples that were flooded when collected were maintained under anaerobic conditions during transport and once in the lab were processed under a H₂/N₂ atmosphere in a glove box (Coy Laboratory Products Inc., Grass Lake, MI).

4.2.2 Soil Chemical analysis

Soil moisture content was determined gravimetrically by drying a 5 g of aliquot of each soil

sample to a constant mass at 80° C in a drying oven. Soil pH was measured using a pH electrode in a 2:1 deionized water:soil slurry solution. Soils were analyzed for total P (TP) by digesting 1.0 g of soil with H₂SO₄ and lithium sulfate-selenium-hydrogen peroxide for 2.5 h at 360° C (Parkinson and Allen 1975). Digests were analyzed for TP colorimetrically using a Bran+Luebbe Autanalyzer 3 (Bran+Luebbe Method # G-103-93, SEAL analytical, Mequon, WI). Microbial biomass P was determined on subsamples of each homogenized soil sample by the chloroform-fumigation direct extraction technique (Brookes et al. 1982). Duplicate 0.5 g sub-samples of soil were taken from each sample. One sub-sample was extracted directly with 0.5M NaHCO₃ after 16 hours of shaking. The second sample was fumigated with CHCl₃ in a tightly capped centrifuge tube overnight, and then extracted with NaHCO₃. Total P was determined for both samples, and microbial biomass P (MicP) was calculated as the difference between the fumigated sample and the non-fumigated sample. For total soil C (TC) and N (TN), subsamples of each soil sample were dried at 80 ° C to a constant mass and then finely ground with a mortar and pestle. Approximately 0.10-0.12 mg of each ground sample were analyzed for total C and total N using a Carlo Erba NA 1500 C/N analyzer (CE Elantech Inc., Lakewood, NJ).

4.2.3 Genomic DNA Isolation and PCR amplification of 16S and 18S-ITS rDNA genes

Total genomic DNA was extracted from 10 g soil subsamples using the Mo-Bio PowerMax soil DNA isolation kit (Mo Bio Laboratories, Solana Beach, CA), according to the manufacturer's instructions, to give a final volume of 100 µL of genomic DNA per sample. Yield and purity of total genomic DNA extractions were estimated on agarose gels (1.5 % w/v) with 0.5 µg mL⁻¹ ethidium bromide and by measuring absorbance at 260/280 nm using UV/VIS

spectrophotometry (Nanodrop 1000, Thermo Scientific, Wilmington, DE). Total soil genomic DNA was extracted from all soil samples individually. The resulting DNA preparations were either pooled for PCR and plasmid cloning to provide full-length sequence information for detecting dominant organisms, or analyzed separately by PCR-DGGE to provide community profile of all organisms present in addition to screening for the presence – absence of dominant organisms in soil samples. For each study, pooled genomic DNA was prepared by combining 250 μ L of genomic DNA from individual soil samples either by site (in Study 1, WT vs. BL) or microsite elevation across sites (in Study 2, RD vs. SW). Therefore the total number of pooled genomic DNA preparations for establishing the plasmid clone library was two in both studies 1 and 2.

Approximately 10 ng of extracted genomic DNA from individual soil samples or pooled DNA preparations of multiple soil extracts (as described above) served as a template for PCR-amplification of internal fragments of the bacterial 16S rDNA and fungal 18S rDNA and ITS regions. Universal bacterial (63-F and 1387-R) and fungal (ITS1-F and ITS4-R) primers were used to PCR-amplify as broad a range of bacterial or fungal taxa from soils, respectively, and to produce near full-length bacterial 16S rDNA (~ 1300 bp) and fungal 18S rDNA/ITS (~ 800 bp) amplicons. The products of five PCR reactions per individual soil sample or pooled DNA preparation were combined and purified using the Mo Bio UltraClean PCR Clean-Up kit (Mo Bio Laboratories, Solana Beach, CA) according to the manufacturer's instructions. These purified full-length amplification products were used as template (2 μ L) in a nested PCR (using bacterial 341F/534R and fungal ITS1F/ITS2 primer) to produce shorter products (~ 200 bp for

bacteria and ~ 300 bp for fungi) suitable for DGGE analysis. Primers 341-F and ITS1-F were synthesized with a 5' 40-bp GC clamp to stabilize the melting behavior of the PCR products analyzed using DGGE (**Table 4.2**).

All PCR reactions were performed under sterile conditions (PCR hood, ESCO Airstream PCR Station, Hatboro, PA) in autoclaved and UV irradiated 0.2 mL tubes (TKR Biotech Products, Huntington Valley, PA) using a Hybaid PCR-Express 2 (Hybaid USA, Franklin, MA) thermal cycler. The reaction mixture (final volume of 50 μ L) contained 25 μ L of PCR Master Mix (Promega Corp., Madison, WI), 1.0 μ L of each primer (20 pmol final concentration), and 21 μ L of molecular grade nuclease-free water (Promega Corp., Madison, WI). Negative controls were included with all PCR reactions by substituting 2.0 μ L nuclease-free water for template DNA. In nested PCR reactions additional negative control tubes from the first stage were diluted 1/10 for use as template in second stage (nested PCR) negative controls. Positive controls used molecular grade DNA extracts from either *S. cerevisiae* or *E. coli* (Sigma-Aldrich, St. Louise, MO) for the template. Only PCR reactions giving the expected results in all controls were used. All PCR products were visualized on agarose gels (1.5 % w/v) with 0.5 μ g mL⁻¹ ethidium bromide and quantified by measuring absorbance at 260/280 nm using UV/VIS spectrophotometry (Nanodrop 1000, Thermo Scientific, Wilmington, DE).

4.2.4 Cloning, Sequencing, and Phylogenetic Analysis of 16S and 18S-ITS rDNA genes

Composite PCR products of near full-length bacterial 16S rDNA (~ 1300 bp, primers 63-F and 1387-R) and fungal 18S rDNA/ITS (~ 800 bp, primer ITS1-F and ITS4-R) amplifications from pooled DNA preparations of multiple soil extracts (as described above for each study), were cloned into plasmid vectors pCR 2.1 using a TOPO TA Cloning kit (Invitrogen Corp., Carlsbad, CA), following the manufacturers protocol. White colonies were subcultured in Terrific Broth containing kanamycin (96 well plates), incubated overnight at 35 °C, and the plasmids harvested using an alkaline lysis technique (SeqPrep, EdgeBiosystems, Gaithersburg, MD), following the manufacturers protocol. Purified plasmid-inserts were sequenced with primers 63-F for the bacterial and ITS4-R for the fungal library using the BigDye Terminator Cycle Sequencing Kit on an automated DNA sequencer (ABI Model 3130, Applied Biosystems, Foster City, CA).

Conserved motifs of near-full length bacterial 16S rDNA or fungal 18S rDNA-ITS, were identified independently for each study, and the sequences were trimmed (of vector contamination) to ensure that all sequences had the same start and end point. Chimeric sequences resulting from PCR artifacts were identified using the online program Check-Chimera from the Ribosomal Database Project II (<http://rdp8.cme.msu.edu>) and removed prior to subsequent analyses. Pair-wise similarity values were generated for all bacterial or fungal sequence libraries in each study using the online program Needle in the Emboss software suite (<http://www.ebi.ac.uk>). Operational taxonomic units (OTU) were defined as a group of sequences sharing $\geq 97\%$ similarity within bacterial or fungal sequence libraries in each study. Representative sequences of each OTU were submitted to GenBank using the Blastn search

engine, and the resulting subjects, along with the queries, aligned by the automated multiple alignment software ClustalX 2.0.11 (European Molecular Biology Network). Final bacterial or fungal alignments for each study were used to perform phylogenetic tree searches using the maximum-parsimony method in PAUP 4.0b10. Maximum-parsimony analysis was performed under heuristic search methods using tree bisection-reconnection (TBR), and 1000 random, stepwise addition replicates.

4.2.5 Denaturing gradient gel electrophoresis

The DGGE (Muyzer et al. 1993) analysis was performed on a D-Code Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA). Composite PCR products (~ 500 ng) were loaded on polyacrylamide gels (8 %) prepared with denaturing conditions that were optimized based on preliminary results, either 45 – 65 % for bacterial or 30 – 50 % for fungal PCR products, where 100 % denaturant is defined as 7 M urea and 40 % v/v formamide. Electrophoresis was performed in tris-acetate buffer at 75 V and 60 °C for 16 hr. Gels were stained with 0.5 µg mL⁻¹ ethidium bromide and visualized with UV trans-illumination using gel documentation software (Quantity One, Bio-Rad Laboratories, Hercules, CA). A selection of clone sequences representing some of the dominant OTUs retrieved from the clone-library of each study were reamplified with bacterial 341F/534R or fungal ITS1F/ITS2 DGGE primers and pooled to construct reference standards. These reference standards (M1 – M2) were loaded on DGGE gels flanking soil DNA-PCR products, to help with the identification of prominent DGGE bands in the environmental samples and serve as external standards when combining and anchoring (normalization) multiple gels for densitometry analysis.

Amplicons of interest that generated prominent DGGE bands were excised and selected for cloning and sequencing. Dominant bands were excised from DGGE gels, and acrylamide slices were crushed and resuspended overnight at 4 °C in 20 µL of sterile water to elute the DNA and subsequently used as templates for PCR using the bacterial or fungal DGGE primers as described above. Amplicons of excised bands were re-run on steeper DGGE gradient gels against the initial soil DNA-PCR sample to ensure purity, co-migration, and that the target band had been amplified. Re-amplified PCR products showing single bands during DGGE analysis were purified, cloned, and the inserts sequenced as described above with either the bacterial 534-R or fungal ITS2-R primer. Similarity (> 97 %) between dominant clone-library sequences and sequences derived from prominent DGGE bands were analyzed using Needle in the Emboss software suite.

4.2.6 Soil chemical and DGGE profile data analysis

The DGGE banding patterns were converted to qualitative and quantitative matrices using GelCompar II v6.5 (Applied Maths, Inc., Austin, TX) to facilitate comparison of the soil samples in a gel profile. Two tables were constructed (with samples as rows and DGGE bands as columns): 1) a qualitative binary table where bands were scored as present (score = 1) or absent (score = 0); and 2) a quantitative analysis that took into account the relative intensity of each band in a sample (or DGGE lane). The matrix generated by calculating the relative intensity of bands was used for further analysis to eliminate the bias caused by any minor differences in PCR product amount loaded in the gels (Salles et al. 2004). Band intensities (peak area) were

normalized by dividing each band's absolute intensity by the total intensity of bands per lane. Diversity indices (Shannon-Wiener index, ***H***) were calculated from the presence/absence and relative band intensities per sample using the following equation:

$$H = \sum_{i=1}^S (P_i)(\ln P_i),$$

where ***S*** is the number of species in the sample and ***P_i*** is the relative intensity of each individual band divided by the total intensity of bands per lane. Evenness (***E***), a measure of how similar the abundances of different OTUs was calculated as $E = H/\log(S)$.

Dendrograms were constructed (using GelCompar II v6.5 software) by the Jaccard correlation coefficient (***S_j***) calculated for each pair of samples (DGGE lanes) within a gel, and cluster analysis was performed by the unweighted pair group method with average linkages (UPGMA), to determine if OTU similarities resulted in clustering patterns that could be related to floodplain type, study site, and/or topographic variation in microbial communities. The similarity of two samples was assessed using the presence/absence and relative intensities in banding patterns using the following equation:

$$S_j = n_{AB} / (n_A + n_B) - n_{AB},$$

where ***n_{AB}*** is the number of bands common to both samples (lanes) **A** and **B**, ***n_A*** is the total number of bands in sample **A**, and ***n_B*** is the total number of bands in sample **B**.

Significant differences in soil pH, moisture, total C, N, P, and microbial P at each site were determined using a two-way ANOVA ($\alpha = 0.05$), with main effects of floodplain type (AL vs. BW) and microsite elevation (RD vs. SW), with site nested within floodplain type, and the interaction terms. Data were log transformed where necessary to meet the assumption of normality. For each study, to correlate differences in chemical characteristics and microbial community structure in these soils, a multivariate statistical analysis was applied to the matrix containing the relative intensity values of DGGE bands (OTUs). The model underlying the relationships among the bacterial and fungal community compositions (DGGE banding patterns) and the soil chemical variables is often written as:

$$\mathbf{Y} = \mathbf{XB} + \mathbf{ZC} + \mathbf{E},$$

where $\mathbf{Y}_{(n, p)}$ represents the community composition data for the p species (bacterial or fungal bands, OTUs), $\mathbf{X}$ represents the design matrix for the factors relating to floodplain type and/or topography, $\mathbf{Z}_{(n, q)}$ represents the q soil chemical characteristics, and $\mathbf{E}$ represents the $n \times p$ error matrix. Classically, the problem reduces to estimating the parameter matrices $\mathbf{B}$ and $\mathbf{C}$ based on appropriate error terms. Unfortunately, the classical model does not hold for the following reasons (Harner, J.E. *personal communication*):

- The number of OTUs is large and is greater than the number of observations, i.e., $p > n$. As a result, classical estimation theory does not work.
- The DGGE gel images (analyzed with GelCompar II v 4.06) are coded by the relative intensities (Dinsdale et al. 2008) of each band (j) within the i^{th} soil profile. Therefore, $\sum y_{ij} = 1$, i.e., the total intensities for each profile sum to 1 and the species compositional data matrix $\mathbf{Y}$ is singular.

- A high proportion of intensities are 0 for some OTUs.
- The components of the error matrix **E** are not normal.

The approaches in the literature (e.g., Fromin et al. 2002, Ramette 2007) have fallen short on a number of fronts, e.g., the analyses have been restricted to just the presence/absence of OTUs (**Y**) and environmental data (**Z**). These analyses have generally taken two approaches: 1) relating the separate **Y** and **Z** matrix decompositions, e.g., principal component analysis or correspondence analysis, in an *ad hoc* manner; and 2) using canonical correspondence analysis or redundancy analysis to directly relate the chemistry variables to the species variables (Fromin et al. 2002). Generally, the intensity data have been reduced (with a loss of information) to presence (1) / absence (0) binary data to remove the compositional restriction, i.e., $y_{ij} = 0$ or 1 for all i and j (Ramette 2007).

The principal hypotheses in this work are to relate the effects of the floodplain type and/or microsite elevation to species (or OTU) composition (relative intensity of bands), after adjusting for soil chemical effects. Due to the requirements of this project and the analytical difficulties listed above, I used detrended correspondence analysis (DCA – *decorana* function) as implemented in the Vegan 2.0 (Oksanen et al. 2011) package for R v. 2.13. The *envfit* and *ordiellipse* functions were used to create a secondary matrix, from which the soil chemical variables (spatially visualized as vectors) were combined with the band intensity ordinations. The data matrices containing relative band intensities were $\log(x + 1)$ transformed before analysis. The significance ($P \leq 0.05$) of correlation between explanatory variables and microbial community composition are based on 999 random permutations of the data. Confidence ellipses

(*ordiellipse*) at the 0.95 level for soil samples were included in ordinations to examine the variability of floodplain type, site, and/or topographic variation within each ordination.

4.3 RESULTS

4.3.1 Soil chemistry

Soil pH across RD and SW microsite elevations in the BL and WT floodplain sites (Study 1) ranged from 5.1 to 6.1, averaging 5.4 in BW and 5.6 in AL soils (**Table 4.3**). Other factors important to microbial growth, including TC (averaged 596 kg ha⁻¹ in BW and 261 kg ha⁻¹ in AL soils) and soil moisture (averaged 45 % in BW and 29 % in AL soils) content were significantly higher in the BW soils, whereas soil TP content (averaged 4 kg ha⁻¹ in BW and 12 kg ha⁻¹ in AL soils) was significantly higher in the AL FF (**Table 4.3a,b**). Total C, TN and moisture content were significantly higher in topographic lows (SW soils) compared to RD soils.

Across multiple FF sites (Study 2), soil TC content (averaged 811 kg ha⁻¹ in BW and 298 kg ha⁻¹ in AL soils) was significantly higher in BW sites; soil TP (averaging 4 kg ha⁻¹ in BW and 9 kg ha⁻¹ in AL soils) was significantly higher in AL floodplain soils (**Table 4.4a,b**). Moisture content was significantly higher in SW compared to RD soils in both AL and BW FFs. In both Study 1 and 2, microbial biomass P averaged 0.1 kg ha⁻¹ in both AL and BW floodplain soils, and did not differ significantly in AL versus BW or RD versus SW soils (**Tables 4.3, 4.4**).

4.3.2 Clone library construction and phylogenetic distribution of clones

4.3.2.1 Description of libraries across microsite elevations in an AL and a BW floodplain site

In study 1, a total of 399 non-chimeric 16S rDNA bacterial sequences comprised 187 unique OTUs, and were placed in 10 major taxa: *Alpha-*, *Beta-*, *Gamma-*, *Deltaproteobacteria*, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Chloroflexi*, and *Bacteroidetes*. *Acidobacteria* and *Alphaproteobacteria* were the most prevalent groups based on sequence analysis, accounting for 45 and 28 % of the library, respectively (**Figure 4.1a; Appendix Table 4.1a**). A total of 320 fungal ITS sequences comprised of 147 unique OTUs placed in 4 major taxa: *Ascomycota*, *Basidiomycota*, *Zygomycota*, and *Chytridiomycota*. The majority of fungal sequences belonged to the *Basidiomycota* (47 %) and *Ascomycota* (45 %) divisions (**Figure 4.1b; Appendix Table 4.1b**).

4.3.2.2 Description of libraries in four AL and four BW FFs

In Study 2, a total of 448 non-chimeric 16S rDNA bacterial sequences comprised 320 unique OTUs, and were placed in 10 major taxa: *Alpha-*, *Beta-*, *Gamma-*, *Deltaproteobacteria*, *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Chloroflexi*, *Nitrospirae*, and *Bacteroidetes*. Similar to Study 1, *Acidobacteria* (43 %) and *Alphaproteobacteria* (26 %) were the most prevalent groups (**Figure 4.2a; Appendix Table 4.2a**). A total of 377 fungal ITS sequences comprised of 114 unique OTUs placed in the 4 major taxa outlined above, and a majority of fungal sequences belonged to the *Ascomycota* (51 %) and *Basidiomycota* (40 %) divisions (**Figure 4.2b; Appendix Table 4.2b**).

4.3.3 Bacterial and fungal community analysis using PCR- DGGE

4.3.3.1 Bacterial community composition in DGGE profiles

A total of 27 and 30 dominant bands were successfully excised, cloned, and sequenced from the DGGE profiles representing study 1-TB (topographic variation in soil one AL and one BW site) and study 2-MB (four AL and four BW floodplain sites), respectively (**Figures 4.3, 4.5, Tables 4.5a, 4.6a**). A majority of the bacterial bands sequenced from the study 1-TB and study 2-MB gels belong to the *Acidobacteria* (47 and 53 %, respectively) and *Alphaproteobacteria* (30 and 27 %, respectively) subdivisions.

4.3.3.2 Fungal community composition in DGGE profiles

A total of 34 and 26 dominant bands were successfully excised, cloned, and sequenced from the DGGE profiles representing Study 1-TF (topographic variation in one AL and one BW site) and Study 2-MF (four AL and four BW floodplain sites), respectively (**Figures 4.4, 4.6; Tables 4.5b, 4.6b**). A majority of the fungal bands sequenced from the study 1-TF and study2-MF gels belong to the *Basidiomycota* (62 and 50 %, respectively) and *Ascomycota* (32 and 38 %, respectively) subdivisions.

4.3.3.3 Diversity, evenness, and UPGMA-Jaccards similarity analysis of DGGE gels.

Diversity (*H*), number of OTUs or bands (*S*), and evenness values were not statistically significant between floodplain type or by microtopographic elevation in most DGGE-gels, with the exception of the fungal DGGE profile in study 1-MF, where *H* and *S* were significantly

higher in AL compared to BW soils (**Table 4.7**).

UPGMA-Jaccards similarity dendrograms generated from presence/absence and relative intensity of DGGE bands, returned similar evaluations of floodplain type patterns on soil bacterial and fungal community composition (**Figures 4.7a,b** and **4.8a,b**). The two main clusters detected in the analysis of all gels in both Study 1 and 2, separated both bacterial and fungal community composition profiles by floodplain type (AL versus BW).

4.3.3.4 Multivariate analysis to correlate microbial community composition in DGGE profiles with soil chemical properties

For both bacterial and fungal communities, detrended correspondence analysis (DCA) resulted in a clear sorting of soil samples by floodplain type (AL versus BW) (with 95 % confidence levels) rather than by microsite elevation (**Figures 4.9 a,b**, and **4.10 a,b**). In this indirect ordination technique, the variations in microbial community structure are derived from the OTU (DGGE band) data independently of the environmental variables. In the DCA of the microbial community structure of Study 1, the first two axes explained 69 and 19 % of the bacterial and 72 and 58 % of the fungal OTU distribution in soils, and show a clear separation between AL and BW soil communities ($p = 0.001$, **Figures 4.9a,b**). Similarly in Study 2, eigenvalues for the first two axes explained 23 and 17 % (bacterial) and 66 and 53 % (fungal) of the community variation in soils, differentiating soil communities by both floodplain type ($p = 0.001$) and site ($p = 0.001$, **Figures 4.10a,b**). In both studies, topographic variations in soil bacterial and fungal community profiles were not statistically significant in all DCA ordination results.

Correlating soil chemistry with DCA ordinations of the microbial profiles was achieved by fitting soil pH, moisture content, TC, TN, TP, and microbial P vectors onto the ordination space using the *envfit* function in Vegan. Fitting of soil chemical variables onto ordinations revealed that TP and TC correlated significantly ($P < 0.05$) with bacterial and fungal community profiles in both studies 1 and 2 (**Figures 4.9a,b** and **4.10a,b**). When comparing soils collected from the BL and WT floodplain sites (Study 1; **Figure 4.9b**), soil pH was significantly ($P < 0.011$) correlated with the fungal community ordination. In vector fitting, a soil chemical variable is represented in the resulting ordination plot by an arrow that points in the direction of the most rapid change in that variable, and the length of the arrow being proportional to the correlation between microbial community ordination and the soil chemical variable (Oksanen et al. 2011). In both Study 1 and 2, the subsequent fitting of variable onto the ordination revealed that TP was associated with AL FFs, whereas TC was significantly correlated with BW FFs (**Figures 4.9a,b** and **4.10a,b**). In the one instance where soil pH was significantly correlated with the fungal community ordination (**Figure 4.9b**), variation in soil pH was associated with changes in fungal community structure in AL floodplain soils.

4.4 DISCUSSION

4.4.1 Microbial community composition in FF soils – clone library analysis

Fungal ITS sequences isolated from Study 1-TF and Study 2-MF represented 147 and 114 unique OTUs, respectively, belonging to *Ascomycota*, *Basidiomycota*, *Zygomycota*, and *Chytridiomycota* divisions (**Figures 4.1b, 4.2b; Appendix Tables 4.1b, 4.2b**). The majority of fungal sequences belonged to the *Ascomycota* (45 % in the TF and 51 % in the MF libraries) and *Basidiomycota* (47 and 40 %, respectively) divisions. Bacterial 16S rDNA sequences isolated from Study 1-TB and Study 2-MB represented 187 and 320 unique OTUs, respectively, belonging to *Alpha*-, *Beta*-, *Gamma*-, *Deltaproteobacteria*, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Chloroflexi*, *Nitrospirae*, and *Bacteroidetes* groups (**Figures 4.1a, 4.2a; Appendix Tables 4.1a, 4.2a**). A similar bacterial community structure was detected in soils collected from a small-scale floodplain adjacent to the Flathead River in Northwestern Montana using DGGE analysis (Lowell et al. 2009). In the current study, *Acidobacteria* (45 % in the TB and 43 % in the MB libraries) and *Alphaproteobacteria* (28 % and 26 %, respectively) were the most prevalent groups based on sequence analysis. The predominance of *Acidobacteria* and *Alphaproteobacteria* phyla (OTUs) in bacterial 16S rDNA libraries has been detected in a wide variety of terrestrial soils (i.e., Rosch and Bothe 2009, Kumar and Khanna 2010, Lin et al. 2010, Michel and Williams 2011, Chen et al. 2012b, Zhao et al. 2012), wetland (i.e., Kim et al. 2004, Hartman et al. 2008, Yang et al. 2008, Tian et al. 2009, Ben Said et al. 2010, Sun et al. 2011, Song et al. 2012), and marine (Dang et al. 2009, Li et al. 2009, Polymenakou et al. 2009, Gao et al. 2011) sediments. Identifying the taxonomic diversity of bacteria and fungi in FF soils is the first step towards linking microbial diversity and ecosystem function. As a second step, linking structure to biogeochemical function requires a comprehensive search in the literature to

produce a database identifying the dominant metabolic function of each taxa present (Grimm et al. 2003, Zak et al. 2006, Dinsdale et al. 2008). Although it has been demonstrated that soil biogeochemical function and microbial ecology vary considerably between and even within ecosystems, a comparative assessment of the controls over the distribution and relative abundance of microbial communities across other ecosystems is an important step in determining structure-function relationships within wetlands. In an attempt to associate metabolic function and relevant processes with respect to C, N, P, S, and Fe cycling in wetlands to the taxonomic identity of microbes sequenced in this study, I conducted a broad comparison of the bacterial and fungal taxa with known biogeochemical function(s) in terrestrial, aquatic, and wetland ecosystems in the literature.

4.4.1.1 Organic matter decomposition

Cellulose and lignin are the products of photosynthesis, and their degradation by microbially produced ligno-cellulolytic enzymes represents the major carbon flow from fixed carbon sinks to atmospheric CO₂ in terrestrial and wetland ecosystems (Cullen and Kersten 2004, Thatoi et al. 2012). The *Ascomycota* sequenced in both the TF and MF libraries were primarily of the subdivision *Pezizomycotina* (**Appendix Tables 4.1b, 4.2b**), which are ecologically diverse fungi occurring in aquatic (Jones et al. 2009, Schoch et al. 2009) and terrestrial habitats (Ruibal et al. 2008, Damon et al. 2010, Unterseher and Schnittler 2010, Gottel et al. 2011). *Pezizomycotina* are either saprophytic chemoheterotrophs, capable of obtaining C nutrition by litter/wood decomposition (class: *Dothideomycetes*, *Eurotiomycetes*, *Leotiomyces*, and *Sordariomycetes*), or form endotrophic-mycorrhizal associations with living plants (class: *Dothideomycetes*,

Pezizomycetes, and *Sordariomycetes*) (Spatafora et al. 2006, Edwards et al. 2011). *Dothideomycetes* (order: *Capnodiales* and *Pleosporales*), *Eurotiomycetes* (*Chaetothyriales* and *Eurotiales*), *Leotiomycetes* (*Helotiales*) and *Sordariomycetes* (*Hypocreales* and *Sordariales*) have broadly similar abilities of obtaining C by degrading complex carbohydrates (cellulose, hemicelluloses, pectin, starch) in litter (Midgley et al. 2004, Raja et al. 2010, Badali et al. 2011, Zifcakova et al. 2011). One taxa within *Sordariomycetes* (order: *Hypocreales*; family: *Nectriaceae*) OTU (1-MF-346) was found to dominate clones sequenced from the MF library, accounting for 133 of the total 377 clones isolated in the library (**Appendix Table 4.2b**). Some species of *Nectriaceae* also occur as parasites of trees (Schmidt and Panstruga 2011, Hopkins et al. 2012). A majority of the *Basidiomycota* sequenced in both libraries belonged to the *Agaricomycotina* subdivision (**Appendix Tables 4.1b, 4.2b**). Similar to the *Ascomycota* found in these soils, *Agaricomycotina* (class: *Agaricomycetes* and *Tremellomycetes*) are either saprophytic and/or form mycorrhizal associations with living plants (Dighton et al. 1990, Selosse et al. 2002, Edwards and Zak 2010). *Agaricomycetes* (order: *Agaricales*, *Boletales*, *Corticiales*, *Polyporales*, *Russulales*) contains a large number of saprophytic taxa (Hibbett 2006, Lawrey et al. 2008), producing extracellular ligno-cellulolytic (endocellulase, cellobiohydrolase, glucosidase, endoxylanase, and N-acetylglucosaminidase) enzymes that degrade complex carbohydrates into C and energy sources that are required by these fungi (Gibertoni and Drechsler-Santos 2010). Although proportions of *Zygomycota* isolated in clone libraries accounted for only 7 % of the TF and MF studies (**Figures 4.1b, 4.2b**), a majority of the taxa belonged to the *Mucoromycotina* subdivision and were of the *Mortierellales* order which live exclusively as saprotrophs in soils, contributing to the turnover of soil C by decaying litter and other soil organic matter (Kurakov et al. 2008, Murase et al. 2012).

In soils rich in organic material, the decomposition of cellulose and lignin involves complex communities of interacting microorganisms, including bacteria (Hoffman and Arnold 2010, Huang et al. 2010, DeAngelis et al. 2011). *Acidobacteria* (order: *Acidobacteriales*, *Holophagales*, and *Solibacterales*, **Appendix Tables 4.1a, 4.2a**) possess a large range of extracellular enzymes involved in the solubilization of complex carbohydrates (xylan, cellulose, hemicelluloses, pectin, starch, and chitin) and utilization of C (Ward et al. 2009, DeAngelis et al. 2011, Tang et al. 2011, Eichorst and Kuske 2012, Pankratov et al. 2012, Stursova et al. 2012, Talia et al. 2012). *Alphaproteobacteria* (order: *Caulobacterales*, *Rhizobiales*, and *Rhodospirillales*) are equally diverse in soil biogeochemical processes, including a fundamental role in driving C turnover by decomposing organic matter and solubilizing/utilizing complex carbohydrates (Beloqui et al. 2010, Hutcheson et al. 2011, Lucena et al. 2012, Teeling et al. 2012), some of which are polycyclic aromatic hydrocarbons that are toxic compounds in soils/sediments (Schleheck et al. 2000, Kanamori et al. 2002, Rockne and Strand 2003, Kwon et al. 2005, Liu et al. 2011, Chen et al. 2012a).

4.4.1.2 Nutrient uptake by saprophytic and root-rhizosphere associated bacteria and fungi

Saprophytic *Ascomycota*: *Dothideomycetes* (order: *Capnodiales* and *Pleosporales*), *Eurotiomycetes* (*Chaetothyriales* and *Eurotiales*), *Leotiomycetes* (*Helotiales*), *Sordariomycetes* (*Hypocreales* and *Sordariales*) and *Basidiomycota*: *Agaricomycetes* (order: *Agaricales*, *Boletales*, *Corticiales*, *Polyporales*, *Russulales*) (Hibbett 2006, Lawrey et al. 2008), produce extracellular nitrogenases, phosphatases, and arylsulfatases to access insoluble organic N, P, and

S from organic substrates in soil (Delabastide et al. 1995, Zifcakova et al. 2011). In an assessment of biomass P content in common soil fungi, select taxa within *Agaricales*, *Boletales*, and *Russulales* averaged 8.2 g P per kg dry mass; biomass P content was highest (10.7 g P per kg dry mass) in *Agaricales* species (Vetter 1994).

In contrast to saprophytic nutrition, mycorrhizae associated taxa within the *Ascomycota*: *Dothideomycetes* (*Venturiales*), *Sordariomycetes* (order: *Hypocreales*), as well as class *Pezizomycetes* (*Pezizales*) (Spatafora et al. 2006, Edwards et al. 2011) and *Basidiomycota*: *Agaricomycetes* (order: *Agaricales*, *Boletales*, *Russulales*, *Thelephorales*) (Yorou and Agerer 2011, Porter et al. 2008, Gottel et al. 2011) colonize the roots of grasses, herbaceous plants, and some trees, providing N and P to the host, who in return supply C to the associated fungus (Maia et al. 1996, Sanon et al. 1997, Selosse et al. 2002, Dokmetzian et al. 2005, Hoffman and Arnold 2010, Zifcakova et al. 2011). *Tremellomycetes* (order: *Cystofilobasidiales*, *Tremellales*) are teleomorphic and anamorphic yeasts, which are nutritionally heterogeneous fungi comprising saprophytes, plant and fungal endophytes, as well as plant and animal pathogens (Mouhamadou et al. 2011, Takashima et al. 2011, Millanes et al. 2012). A *Tremellomycete* (order: *Tremellales*; family: *Cryptococcus*) OTU, 1-TF-67 and 2-MF-271 was a dominant clone sequenced from both the TF and MF libraries (**Appendix Tables 4.1b, 4.2b**). *Cryptococcus* species have been sequenced in diverse environments including forest soils (Wieferich et al. 2011, Stursova et al. 2012), agriculture soils (Botha 2011, Yurkov et al. 2012), and wetland sediments (Slavikova and Vadkertiova 1997, Rojas et al. 2009, Brandao et al. 2011), and are largely associated with lignocellulolytic activity (Jimenez et al. 1991, Arora and Sharma 2010, Zifcakova et al. 2011) as

well as inorganic N and P solubilization and uptake from soil organic matter and sediment (Greenwood and Lewis 1977, Boby et al. 2008, Cloete et al. 2010, Botha 2011, Singh and Singh 2012).

Acidobacteria (order: *Acidobacteriales*, (**Appendix Tables 4.1a, 4.2a**) isolates are also known to play an important role in iron redox reactions and the uptake and metabolism of iron from various substrates (Rowe et al. 2007, Blothe et al. 2008, Kleinsteuber et al. 2008, Ward et al. 2009). Some taxa within the *Acidobacteria* phylum (primarily *Acidobacteriales* and *Holophagales*) are capable of nitrate and nitrite reduction (Hall et al. 2008, Navarro et al. 2009, Van Trump et al. 2011), but not N-fixation or denitrification (Ward et al. 2009). However a majority of *Rhizobiales* (family: *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, *Methylocystaceae*, *Xanthobacteraceae*) within the *Alphaproteobacteria* subdivision, are symbiotic N-fixers or denitrifiers that have been isolated from the rhizosphere of terrestrial (i.e., Anderson et al. 2011, Chen et al. 2012b, Eichorst and Kuske 2012, Montana et al. 2012) and wetland plants (Valenzuela-Encinas et al. 2009, Jin et al. 2011, Wang et al. 2012). Although *Rhizobiales* are primarily involved in the N cycle, taxa within *Bradyrhizobiaceae* and *Xanthobacteraceae* are capable of utilizing specific enzymes for the solubilization and uptake of phosphate in the rhizosphere (Mikanova and Kubat 1994, Vazquez et al. 2000, Sridevi and Mallaiah 2009, Dodd et al. 2010, Hayat et al. 2010, Palaniappan et al. 2010, Zafar et al. 2011, Pooja 2012, Salvador Montana et al. 2012). Although I did not specifically target for the methanogenic microbial community, which are predominantly archaeobacteria, flood induced anaerobic conditions typical of FF soils are ideally suited for methanogenic archaea that are obligate anaerobes. However,

methanotrophic microbes (i.e., *Methylocystaceae*; **Appendix Tables 4.1a, 4.2a**) that utilize methane as their sole C source, many of which are found within the eubacterial kingdom, were captured using the universal 16S rDNA primers in the current study (Knief et al. 2006, Lau et al. 2007, Chen et al. 2008, Kolb 2009, Stein et al. 2011). A majority of the taxa within *Rhodospirillales* are denitrifying or N-fixing, and phosphate-solubilizing organisms, that are either associated with the root-rhizosphere (family: *Acetobacteraceae*) (Loganathan and Nair 2004, Samaddar et al. 2011) or photosynthetic free-living purple non-sulfur bacteria (*Rhodospirillaceae*) (Madigan et al. 1984, Wang et al. 2008, Diaz-Cardenas et al. 2010).

Although the remaining eubacterial subdivisions contribute to a relatively minor component, totaling 27 % of the TB and 31 % of the MB clone libraries (**Figures 4.1a, 4.2a**), these taxa may also play important roles in C, N, and P biogeochemistry of floodplain soils. Many taxa within *Betaproteobacteria* (order: *Burkholderiales* and *Rhodocyclales*), *Deltaproteobacteria* (*Myxococcales*), *Gammaproteobacteria* (*Chromatiales*), and *Actinobacteria* (*Actinomycetales*, *Acidimicrobiales*) (**Appendix Tables 4.1a, 4.2a**) are associated with the solubilization of N and P from organic matter and the metabolism of complex carbohydrates (ligno-cellulolytic activity), polycyclic aromatic hydrocarbons, proteins, and lipids, and produce catalytic enzymes, such as phosphatases, glycosyltransferases, dehydrogenases, methyltransferases, dehydratases and epoxide hydrolases (Nielsen et al. 2002, Tanahashi et al. 2005, Vinas et al. 2005, Anderson et al. 2011, Farina et al. 2012, Montana et al. 2012, Stursova et al. 2012).

Other taxa within *Betaproteobacteria* (order: *Burkholderiales* and *Rhodocyclales*) and *Deltaproteobacteria* (*Desulfuromonadales*) are heterotrophic N-fixing and/or denitrifying bacteria (Xie et al. 2003, Weon et al. 2008, Zhang et al. 2008), whereas *Betaproteobacteria* (*Rhodocyclales*) are capable of chemolithoautotrophic ferric iron oxidation (Sobolev and Roden 2004), and many taxa within *Actinobacteria* (*Acidimicrobiales*) and *Deltaproteobacteria* (*Myxococcales*, *Desulfuromonadales*, *Syntrophobacterales*) are syntrophic ferric iron or sulfate reducing bacteria in soils and sediments (Tourova et al. 1998, Bowman and McCuaig 2003, Weber et al. 2006, Da Silva et al. 2007, Chao et al. 2010, Itoh et al. 2011, Schmidtova and Baldwin 2011).

4.4.1.3 Polyphosphate accumulating (micro)-organisms (PAO) in enhanced biological phosphorus removal (EBPR) systems

Many of the bacteria sequenced in Studies 1 and 2 have been identified as efficient phosphate accumulating organisms in EBPR systems. Enhanced biological phosphorus removal is a technology that exploits the microbial (primarily bacterial) removal P from wastewater (Blackall et al. 2002, Hirota et al. 2010, Gebremariam et al. 2011). The removal of P in wastewater effluent EBPR sludge reactor systems is dependent on the capability of select microorganisms to remove phosphate from the liquid phase and convert it to sludge in the form of intracellular polyphosphates (Oehmen et al. 2007, Hupfer et al. 2008, Hirota et al. 2010). These polyphosphate accumulating organisms (PAOs) are capable of luxury uptake/consumption; the accumulation of phosphorus beyond what is required for growth, and storing them in specialized vacuoles within the cell (Serafim et al. 2002, Zheng et al. 2004, Oehmen et al. 2007, Szabo et al.

2011). The most commonly isolated PAOs in EBPR systems are *Acidobacteria* (order: *Acidobacteriales*) (Crocetti et al. 2002, Ahn et al. 2008), and *Rhizobiales* (family: *Hyphomicrobiaceae*) (Zhang et al. 2011) and *Rhodospirillales* (*Acetobacteraceae*, *Rhodospirillaceae*) (Levantesi et al. 2002, Wong et al. 2004, Wong and Liu 2007, Nittami et al. 2009, Zhang et al. 2011) within the *Alphaproteobacteria* (Wilmes and Bond 2006, Gu et al. 2008, Lopez-Vazquez et al. 2009a, Lanham et al. 2011, Ren et al. 2011, Sanchez et al. 2011) subdivision, and possess large poly-phosphate containing vacuoles within their cytoplasm, thus are well adapted for the luxury consumption of phosphate from wastewater effluent. With the assumption that the TB and MB libraries were dominated by a similar functional diversity of *Acidobacteria* and *Alphaproteobacteria* taxa (**Appendix Tables 4.1a, 4.2a**), these organisms may be the most efficient at P removal at the interface between floodwaters and the soil surface in FFs. Although the remaining subdivisions are relatively minor contributors to the overall community composition of FF soils in this study, *Betaproteobacteria* (order: *Burkholderiales*, and *Rhodocyclales*) (Ruiz-Lozano and Bonfante 1999, Mullan et al. 2002, Wu et al. 2009, Datta and Goel 2010, Zhang et al. 2011), *Gammaproteobacteria* (*Xanthomonadales* and *Chromatiales*) (Kong et al. 2006, Martin et al. 2006, Kim et al. 2011), *Actinobacteria* (*Actinomycetales* and *Acidimicrobiales*) (Kong et al. 2005, Beer et al. 2006), *Bacteroidetes* (*Flavobacteriales*, *Sphingobacteriales*, and *Cytophagales*) (Kawaharasaki et al. 1999, Liu et al. 2001, Liu et al. 2005), and *Nitrospirae* (Nielsen et al. 2010, Onnis-Hayden et al. 2011) taxa accumulate P and have been isolated from functioning EBPR systems. A fungal species found to dominate the TF and MF libraries (*Tremellomycete*, order *Tremellales*, family: *Cryptococcus*) in the current study has also been isolated from sewage granules, has been found to contribute to polyphosphate utilization from wastewater in an aerobic sewage treatment plant, and is regarded

as useful in microbial biofilm formation in EBPR systems (Kacprzak and Stanczyk-Mazanek 2003, Weber et al. 2009).

Although EBPR systems are well established in Australia and Europe, here in the US functional reactor plants remain operationally unstable, primarily because of a lack of understanding of the microbiology of these EBPR systems (Hupfer et al. 2008, Hirota et al. 2010, Gebremariam et al. 2011). Efforts to establish EBPR reactors in the US have largely been seeded and cultured with mixed PAOs imported from European systems (Zheng et al. 2004, Oehmen et al. 2007, Gebremariam et al. 2011). An alternative strategy would be to use PAOs that are native to US soils which are adapted to changing environmental and/or operational factors, including seasonal changes in temperature, pH, anaerobic-aerobic contact time, and organic loading rate, and may increase the success rate of developing EBPR systems in the US (Lopez-Vazquez et al. 2009b). Similar efforts have been successful in establishing large-scale EBPR batch reactors by culturing PAOs isolated from regional wetland sediments and forest soils (Krishnaswamy et al. 2009, Nielsen et al. 2012).

4.4.2 Bacterial and fungal PCR-DGGE profiles

A majority of the bacterial bands excised, cloned, and sequenced from Study 1-TB (topographic variation in soil from one AL and one BW site) and Study 2-MB (four AL and four BW floodplain sites) belong to the *Acidobacteria* (47 and 53 %, respectively) and *Alphaproteobacteria* (30 and 27 %, respectively) subdivisions (**Table 4.5a**). The fungal bands

successfully sequenced from the study 1-TF and study 2-MF gels were predominantly *Basidiomycota* (62 and 50 %, respectively) and *Ascomycota* (32 and 38 %, respectively) (**Table 4.5b**). Similar trends in dominant OTUs were also observed in the clone-library analysis of pooled soil DNA samples (**Appendix Tables 4.1, 4.2**), substantiating the effectiveness of combining DGGE fingerprint analysis with the plasmid cloning method (i.e., Dias et al. 2011, Knapp et al. 2011, Gorra et al. 2012, Perez-Leblic et al. 2012, Yi et al. 2012, Zhou et al. 2012).

Similar to trends seen with other PCR-based metagenomic assessments of soil microbial ecology (i.e., von Wintzingerode et al. 1997, Amann and Ludwig 2000, Forney et al. 2004, Nocker et al. 2007), average bacterial diversity estimates (Shannon diversity, H) were generally higher than fungal diversity in AL and BW soils (**Tables 4.7, 4.8**). There were few differences in the absolute numbers of bands (OTUs) or Shannon diversity and evenness estimations (presence/absence of bands) between topographic relief (RD vs. SW, for bacteria and fungi in both studies) or floodplain type (AL vs. BW, for bacteria in TB and MB gels and the fungal TB gel), with the exception of the fungal DGGE profile in the MF study. Both the number of OTUs ($p = 0.009$, $F = 8.250$) and diversity values ($p = 0.007$, $F = 8.880$) were significantly higher in AL compared to BW soils in the MF DGGE profile. Although only a few differences were detected by estimating diversity using the presence/absence of bands, the inclusion of relative intensity measurements for each band (estimating relative abundance or dominance of OTUs) and Jaccards similarity coefficient, generated UPGMA dendrograms showing two distinct clusters in the microbial community structure (**Figures 4.7, 4.8**). Both the bacterial and fungal DGGE profiles were clearly separated by floodplain type (AL vs. BW) in Studies 1 and 2, but

there were no divergent clusters separating the microbial community by variations in microsite elevation. The ability for DGGE fingerprinting in combination with UPGMA-Jaccards similarity analysis to discern complex bacterial and/or fungal profiles in environmental samples has been well documented (i.e., Clegg et al. 2003, Kang and Mills 2003, Knupp et al. 2009, Bouskill et al. 2010, Nadarajah et al. 2010, dos Santos et al. 2012).

4.4.3 Multivariate analysis correlating DGGE profiles with differences in TC and TP content in AL vs. BW floodplain soils

Using DCA to examine variations in microbial community composition (presence/absence and relative intensity of DGGE bands) produced a clear sorting of soil samples according to floodplain type (with 95 % confidence levels) for both the bacterial and fungal communities but not according to microsite elevation (**Figures 4.9, 4.10**). Total soil C content was significantly higher in BW than in AL soils (ANOVA **Tables 4.3 , 4.4**), averaging 596.2 ± 77.2 and 811.4 ± 43.3 kg ha⁻¹ in BW and 261 ± 17.0 and 298.4 ± 46.9 kg ha⁻¹ in AL soils measured in Studies 1 and 2, respectively. Total soil P content in Studies 1 and 2, however, was significantly higher in AL (11.6 ± 0.3 and 8.7 ± 1.1 kg ha⁻¹, respectively) compared to BW (4.4 ± 0.2 and 4.4 ± 0.9 kg ha⁻¹, respectively) FF soils. These results are supportive of the trend that AL floodplain soils have higher TP contents, primarily due greater inputs of P with sediment deposition compared to BW systems, while BW floodplain soils typically have higher TC contents than AL soils (Lockaby and Walbridge 1998, Hupp et al. 2005, Noe and Hupp 2005). Soil TN concentrations did not differ significantly by floodplain type (**Tables 4.3b, 4.4b**). Craft and Chiang (2002) reported a critical C:N ratio of 20:1 to indicate N availability for microbial processes. In the

current study C:N ratios of AL and BW floodplain soils were 17:1 and 12:1, respectively, suggesting sufficient N availability for microbial productivity. Soil N:P ratios were significantly higher in BW (N:P = 13.6) compared AL (N:P = 3.4) FF (ANOVA $P < 0.0001$, $F = 45.4367$), suggesting that BW floodplain soils may either be P limited or co-limited by both N and P (Lockaby and Walbridge 1998).

Multivariate statistical analyses correlating soil chemistry (pH, moisture content, TC, TN, TP, and microbial P) with the DCA ordination analysis representing DGGE community profiles (using the *envfit* function in Vegan) revealed that TP and TC were the most influential parameters associated with observed differences in AL vs. BW bacterial and fungal soil community structure (**Figures 4.9, 4.10**). Similar to ANOVA results (**Tables 4.3, 4.4**), the subsequent fitting of environmental variables onto ordination plots revealed that TP was associated with the diversity and composition of bacterial and fungal communities in AL FF soils, while TC was significantly correlated with the microbial community profiles in BW FFs. The effect of TP and/or TC on the diversity and composition of bacterial or fungal communities has been previously reported in terrestrial (van der Heijden et al. 1999, Corbett and Anderson 2006, Edwards et al. 2006, Xiong et al. 2010) and wetland (Garono et al. 1996, Olapade et al. 2005, Zeng et al. 2009, Cao et al. 2011, Kolmonen et al. 2011, Zhao et al. 2011, de Figueiredo et al. 2012) soils and sediments. Similar to trends seen with soil N:P content, C:P ratios were significantly higher in BW (C:P = 234.0) than in AL (N:P = 36.2) FF soils (ANOVA $P < 0.0001$, $F = 45.4367$), and also indicative of the higher likelihood that BW soils are P limited than AL floodplain soils. Tezuka (1990) suggested that the C:P ratio of substrate determines if P limits microbial productivity, and demonstrated that P immobilization rates were high when substrate

C:P ratios are < 40 , but decreased abruptly with increasing substrate C/P concentrations, until P uptake rates reached zero when C:P ratio > 60 . Although in the current study, P incorporated in soil microbial biomass (fumigation experiment) averaged 0.1 kg ha^{-1} and did not differ significantly by floodplain type (**Tables 4.3, 4.4**), soil TP and C:P ratios did suggest differences in P availability, with larger soil P pools in AL than in BW FF soils. These results could indicate that microbial communities in AL floodplains are more efficient at phosphate immobilization (Kairesalo et al. 1995, Bridgham and Richardson 2003, Schultz and Urban 2008, Roberts et al. 2012), while mineralization associated with the decay of C-rich organic matter may dominate microbial processes in BW floodplain soils (Jana et al. 2001, Schultz and Urban 2008, Webster et al. 2009).

Although TN and soil moisture content differed by microsite elevation and were significantly higher in SW compared to RD soils (ANOVA; **Tables 4.3, 4.4**), multivariate analyses of DGGE profiles were not significantly correlated with either soil moisture or TN content (**Figures 4.9, 4.10**). In both studies, the bacterial and fungal community profiles did not statistically differ (DCA analysis) between microsite elevations, and were not significantly correlated with any soil chemical parameter measured. Seasonal flooding events have been shown to increase P availability in low-lying SW microsites (Mitsch and Gosselink 2000; Stoeckel and Miller-Goodman 2001), producing conditions that favor soil microbial communities dominated by obligate anaerobic organisms (Stoeckel and Miller-Goodman 2001) and suggesting that swales may function as ‘hotspots’ of P retention and transformation (Wright et al. 2001; Stoeckel and Miller-Goodman 2001). However in the current study, soil TP, microbial biomass P (**Tables 4.3, 4.4**), and bacterial and fungal community structure (**Figures 4.9, 4.10**) did not differ significantly

in RD versus SW soils. This provides a second line of evidence that differences in soil TP (differing only by floodplain type) are a good predictor of microbial community structure in FFs in the southeastern US. Soil pH however, was significantly correlated with the fungal community ordination in study 1 (**Figure 4.14**), and the variation in pH along with TP content in soil was associated with the variation in fungal community structure in AL floodplain soils. Although average soil pH did not differ between AL and BW floodplains, pH measured in individual soils ranged from 5.1 to 6.1, and since microbes vary in their sensitivity to soil pH, minor changes in pH may correspond with substantial differences in fungal community structure. The effect of soil pH significantly contributing to the variation in fungal community composition has been reported previously in terrestrial soils (van der Heijden et al. 1999, Krivtsov et al. 2003, Hamman et al. 2007, Lopes et al. 2011). Similarly, Hartman et al. (2008) reported that soil pH was an important predictor of the variation in bacterial community composition, followed by land use, restoration status, and differences in soil nutrient content across several freshwater wetlands in the North Carolina coastal plain and Florida Everglades.

4.5 CONCLUSIONS

This study provides primary insight into the diversity and composition of dominant bacteria and fungi in FFs of the southeastern US. Results reveal shifts in the composition of bacterial and fungal communities, and the abundance of specific taxonomic groups, with changes in soil C and P content and to lesser extent pH, that may reflect changes in biogeochemical cycling. Phylogenetic analysis of the clone library resulted in diverse OTUs, belonging to *Alpha*-, *Beta*-, *Gamma*-, *Delta*-proteobacteria, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Chloroflexi*, *Nitrospirae*, and *Bacteroidetes* bacterial groups, and *Ascomycota*, *Basidiomycota*, *Zygomycota*, and *Chytridiomycota* fungal divisions. In both the clone library and DGGE fingerprint data a majority of the bacterial sequences belonged to the *Acidobacteria* and *Alphaproteobacteria* groups, and the most prevalent fungal taxa belonged to the *Ascomycota* and *Basidiomycota* subdivisions. As hypothesized (hypothesis 1), average bacterial diversity was higher than average fungal diversity in both the AL and BW soils. The UPGMA-dendrograms generated by calculating Jaccards similarity coefficient of the presence/absence and relative intensity of bands on DGGE profiles show two distinct clusters in the bacterial and fungal community structure. Also as hypothesized (hypothesis 2), both bacterial and fungal DGGE profiles were clearly separated by floodplain type (AL vs. BW), but there were no distinct clusters dividing higher elevation RD from lower elevation SW soils. The composition of dominant bacterial (*Acidobacteria* and *Alphaproteobacteria*) and fungal (*Ascomycota* and *Basidiomycota*) taxa grouped as a function of environmental characteristics (TP, TC, pH) describing the availability of nutrients in FFs. Total soil C content was significantly higher in BW soils, whereas TP content was significantly higher in AL FF soils. Soil N:P and C:P ratios were significantly higher in BW than in AL FF soils, suggesting that BW soils are more likely to

be P limited or co-limited by both N and P. In support of hypothesis 3, the subsequent fitting of environmental variables onto the ordination plots showed that TP and pH were associated with the diversity and composition of bacterial and fungal communities in AL FF soils, while TC was most strongly correlated with the microbial community profiles in BW FFs. Although I had expected the microbial community structure to vary spatially with changes in microsite elevation (hypothesis 4), soil TP, microbial biomass P, and bacterial and fungal community structure did not differ between RD and SW soils. Many of the bacterial and fungal OTUs detected in this study possess diverse metabolic functions associated with soil C, N, S, and Fe cycling in a variety of ecosystems, and may potentially have similar structure-function relationships in FF soils. More importantly, several bacterial taxa (within *Acidobacteria*, *Alpha-*, *Beta-*, *Gamma-proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Nitrospirae* groups) sequenced in floodplain soils have also been detected in EBPR systems and are efficient at the solubilization and uptake of P and the storage polyphosphates. The abundance of these taxa in AL and BW soils, and their ability to efficiently remove P, suggests a link between microbial species composition and P retention and transformation processes in FF soils. The relationship between soil pH and C, P concentrations and composition of dominant microorganisms were consistent across four AL and four BW FFs in the southeastern US. The taxonomic identity of bacteria and fungi in floodplain soils, and their link to P retention and transformation processes, is critical to assessing the sustainability of these ecosystem services; the specific composition of microbial communities may be a useful biological indicator to aid in assessing the successful replication of these processes in wetland creation and/or restoration efforts.

4.6 REFERENCES

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Figure 4.1: Maximum-parsimony tree showing phylogenetic relationships of cloned **(a)** bacterial 16S rDNA, and **(b)** fungal ITS sequences, from pooled DNA in soils collected in an alluvial (AL, Wateree R., SC) and a blackwater (BW, Black R., NC) FF, to related sequences from GenBank. The bacterial tree is rooted with *Aquifex pyrophilus*; the fungal tree is rooted with *Suberites ficus*, *Antipathes elegans*, and *Beroe ovata* as a monophyletic outgroup. Detailed subtrees of each taxonomic group are provided in **Appendix Figures 4.1 , 4.2**. The inset figure shows the proportional distribution of different taxonomic groups in the respective clone libraries.

Figure 4.1 a: Maximum-parsimony tree showing phylogenetic relationships of cloned bacterial 16S rDNA sequences.

Figure 4.1 b: Maximum-parsimony tree showing phylogenetic relationships of cloned fungal ITS sequences.

Figure 4.2: Maximum-parsimony tree showing phylogenetic relationships of cloned **(a)** bacterial 16S rDNA, and **(b)** fungal ITS sequences, from from ridge and swale soils in four alluvial (AL) (Wateree R., SC; Pee Dee R., SC; Nottaway R., VA; Ocmulgee R., GA) and four blackwater (BW) (Black R., NC; Drowning Cr., NC; Satilla R., GA; Salkehatchie R., SC) FFs, to related sequences from GenBank. The bacterial tree is rooted with *Aquifex pyrophilus*; the fungal tree is rooted with *Suberites ficus*, *Antipathes elegans*, and *Beroe ovata* as a monophyletic outgroup. Detailed sub-trees of each taxonomic group are provided in **Appendix Figures 4.3, 4.4**. The inset figure shows the proportional distribution of different taxonomic groups in the respective clone libraries.

Figure 4.2 a: Maximum-parsimony tree showing phylogenetic relationships of cloned bacterial 16S rDNA sequences.

Ascomycota **Basidiomycota**
Chytridiomycota **Zygomycota**

51% 40% 2% 7%

Ascomycota
Basidiomycota
Chytridiomycota
Zygomycota

Figure 4.3: Bacterial 16S rDNA DGGE fingerprint profile of soils collected from one alluvial (AL) (Wateree R., SC) and one blackwater (BW) (Black R., NC) FF. Lanes 2-7 represent ridge (RD) soils, 8-9 dry swales (SWD), and 10-13 flooded swales (SWF), collected from the Black R., NC, FF. Lanes 16-21 represent ridge (RD) soils, and 22-27 dry swales (SWD), collected from the Wateree R., SC, FF. Lanes 1, 15 (M1), 14, and 28 (M2) are reference standards of dominant clones retrieved from the pooled library and re-amplified with primers GC-341-F and 534-R for DGGE analysis. Clone library sequences used to construct standards M1 and M2 are A: 5-TB; B: 4-TB; C: 12-TB; D: 15-TB; E: 12-TB; F: 6-TB; G: 1-TB; H: 2-TB; I: 3-TB; J: 10-TB. Band B was used construct M1 and M2 as an external standard to anchor gels for densitometry analysis. Arrows and numbers mark prominent bands referred to in **Table 4.5a**, that were excised, cloned, and sequenced for comparison with the clone library.

Figure 4.4: Fungal ITS rDNA DGGE fingerprint profile of soils collected from one alluvial (AL) (Wateree R., SC) and one blackwater (BW) (Black R., NC) FF. Lanes 2-7 represent ridge (RD) soils, 8-9 dry swales (SWD), and 10-13 flooded swales (SWF), collected from the Black R., NC FF. Lanes 16-21 represent ridge (RD) soils, and 22-27 dry swales (SW), collected from the Wateree R., SC, FF. Lanes 1, 15 (M1), 14, and 28 (M2) are reference standards of dominant clones retrieved from the pooled library and re-amplified with primers GC-ITS1-F and ITS2-R for DGGE analysis. Clone library sequences used to construct standards M1 and M2 are A: 10-TF; B: 3-TF; C: 12-TF; D: 14-TF; E: 7-TF; F: 4-TF; G: 46-TF; H: 26-TF; I: 22-TF; J: 19-TF; K: 2-TF; L: 15-TF; M: 23-TF; N: 19-TF; O: 12-TF; P: 18-TF; Q: 21-TF. Arrows and numbers mark the prominent bands referred to in **Table 4.5 b** that were excised, cloned, and sequenced for comparison with the clone library.

Figure 4.5: Bacterial 16S rDNA DGGE fingerprint profile of soils collected from four alluvial (WT: Wateree R., SC; PD: Pee Dee R., SC; NT: Nottaway R., VA; and OC: Ocmulgee R., GA) and four blackwater (BL: Black R., NC; DC: Drowning Cr., NC; ST: Satilla R. GA; and SK: Salkehatchie R., SC) FF. Lanes marked RD represent ridge soils, SWD are dry swales, and SWF are flooded swales. Lanes 1 (M1) and 18 (M2) are reference standards of dominant clones retrieved from the pooled library and re-amplified with primers GC-341-F and 534-R for DGGE analysis. Clone library sequences used to construct standards M1 and M2 are a: 4-MB; b: 7-MB; c: 12-MB; d: 19-MB; e: 16-MB; f: 3-MB; g: 5-MB; h: 38-MB; i: 1-MB; j: 2-MB; k: 9-MB; l: 6-MB; m: 32-MB; n: 39-MB. Bands a and g were used construct M1 and M2 as external standards to anchor gels for densitometry analysis. Arrows and numbers mark the prominent bands referred to in **Table 4.6a**, that were excised, cloned, and sequenced for comparison with the clone library.

Figure 4.6: Fungal ITS rDNA DGGE fingerprint profile of soils collected from four alluvial (WT: Wateree R., SC; PD: Pee Dee R., SC; NT: Nottaway R., VA; and OC: Ocmulgee R., GA) and four blackwater (BL: Black R., NC; DC: Drowning Cr., NC; ST: Satilla R. GA; and SK: Salkehatchie R., SC) FFs. Lanes marked RD represent ridge soils, SWD are dry swales, and SWF are flooded swales. Lanes 1 (M1) and 18 (M2) are reference standards of dominant clones retrieved from the pooled library and re-amplified with primers GC-ITS1-F and ITS2-R for DGGE analysis. Clone library sequences used to construct standards M1 and M2 are a: 3-MF; b: 27-MF; c: 2-MF; d: 25-MF; e: 18-MF; f: 12-MF; g: 32-MF; h: 1-MF; i: 48-MF; j: 6-MF; k: 38-MF; l: 22-MF; m: 33-MF; n: 8-MF; o: 4-MF. Band c was used to construct M1 and M2 as an external standard to anchor gels for densitometry analysis. Arrows and numbers mark the prominent bands referred to in **Table 4.6b** that were excised, cloned, and sequenced for comparison with the clone library.

Figure 4.7: Dendrogram showing clustering of (a) bacterial 16S rDNA, and (b) fungal ITS rDNA DGGE fingerprint profiles of soils collected from one alluvial (AL) (Wateree R., SC) and one blackwater (BW) (Black R., NC) FF. Lanes marked RD are ridge soils, SWD are dry swales, and SWF are flooded swales. The UPGMA clustering of DGGE profiles is based on presence/absence and relative intensities of bands as calculated by Jaccard's similarity coefficient. The scale represents percent similarity between samples.

Figure 4.7 a: Dendrogram showing clustering of bacterial 16S rDNA DGGE fingerprint profile

Figure 4.7 b: Dendrogram showing clustering of fungal ITS rDNA DGGE fingerprint profile

Figure 4.8: Dendrogram showing clustering of (a) bacterial 16S rDNA, and (b) fungal ITS rDNA DGGE fingerprint profiles, of four alluvial (WT: Wateree R., SC; PD: Pee Dee R., SC; NT: Nottaway R., VA; and OC: Ocmulgee R., GA) and four blackwater (BL: Black R., NC; DC: Drowning Cr., NC; ST: Satilla R., GA; and SK: Salkehatchie R., SC) FFs. Lanes marked RD are ridge soils, SWD are dry swales, and SWF are flooded swales. The UPGMA clustering of DGGE profiles is based on presence/absence and relative intensities of bands as calculated by Jaccard's similarity coefficient. The scale represents percent similarity between samples.

Figure 4.8 a: Dendrogram showing clustering of bacterial 16S rDNA DGGE fingerprint profile

Figure 4.8 b: Dendrogram showing clustering of fungal ITS rDNA DGGE fingerprint profile

Figure 4.9: Detrended correspondence analysis (DCA) ordination diagram of **(a)** bacteria, and **(b)** fungal communities, associated with environmental variables (soil moisture, TN, TC, TP, microbial P, and pH) in soils collected from one alluvial (AL) (Wateree R., SC) and one blackwater (BW) (Black R., NC) FF. Arrows represent significant ($P \leq 0.05$) correlations between environmental variables and microbial community composition. The inset table shows the results of the DCA analysis of the variation in microbial community structure applied to soil chemical parameters. Significant ordinations ($P \leq 0.05$) are represented by bold and italicized font.

Figure 4.9a: Detrended correspondence analysis (DCA) ordination diagram of the bacterial community structure associated with environmental variables (soil moisture, TN, TC, TP, microbial P, and pH) in soils.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.69	0.19	0.12	0.09
DGGE band intensity–soil chemical variable correlations				
	Axis 1	Axis 2	R ²	P
TP	-0.97	0.23	0.88	0.001
MicP	-0.12	-0.99	0.03	0.684
TN	0.54	-0.84	0.11	0.283
TC	0.85	-0.53	0.52	0.002
pH	-0.84	0.55	0.17	0.124
Moisture	0.90	0.45	0.18	0.131
Floodplain Type			0.86	0.001
Topography			0.032	0.40

Figure 4.9b: Detrended correspondence analysis (DCA) ordination diagram of the fungal community structure associated with

environmental variables (soil moisture, TN, TC, TP, microbial P, and pH) in soils.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.72	0.58	0.50	0.38
DGGE band intensity–soil chemical variable correlations				
	Axis 1	Axis 2	R ²	P
TP	0.99	0.07	0.72	0.001
MicP	0.63	0.77	0.03	0.709
TN	-0.61	-0.79	0.05	0.579
TC	-0.91	-0.41	0.27	0.029
pH	0.91	-0.41	0.33	0.011
Moisture	-0.70	-0.71	0.08	0.400
Floodplain Type			0.48	0.001
Topography			0.01	0.781

Figure 4.10: Detrended correspondence analysis (DCA) ordination diagram of **(a)** bacteria, and **(b)** fungal, communities associated with environmental variables (soil moisture, TN, TC, TP, microbial P, and pH) in soils collected from four alluvial (WT: Wateree R., SC; PD: Pee Dee R., SC; NT: Nottaway R., VA; and OC: Ocmulgee R., GA) and four blackwater (BL: Black R., NC; DC: Drowning Cr., NC; ST: Satilla R., GA; and SK: Salkehatchie R., SC) FFs. Arrows represent significant ($P \leq 0.05$) correlations between environmental variables and microbial community composition. The inset table provides results of the DCA analysis of the variation in microbial community structure applied to soil chemical parameters. Significant ordinations ($P \leq 0.05$) are represented by bold and italicized font.

Figure 4.10a: Detrended correspondence analysis (DCA) ordination diagram of the bacterial community structure associated with

environmental variables (soil moisture, TN, TC, TP, microbial P, and pH) in soils.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.23	0.17	0.12	0.05
DGGE band intensity–soil chemical variable correlations				
	Axis 1	Axis 2	R ²	P
TP	-0.98	-0.18	0.48	0.013
MicP	-0.49	-0.87	0.14	0.370
TN	0.83	-0.56	0.29	0.107
TC	0.88	-0.47	0.64	0.002
pH	0.03	-0.99	0.11	0.498
Moisture	0.36	-0.93	0.25	0.160
Floodplain Type			0.38	0.001
Site			0.86	0.001
Topography			0.03	0.707

Figure 4.10b: Detrended correspondence analysis (DCA) ordination diagram of the fungal community structure associated with

environmental variables (soil moisture, TN, TC, TP, microbial P, and pH) in soils.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.66	0.53	0.50	0.25
DGGE band intensity–soil chemical variable correlations				
	Axis 1	Axis 2	R ²	P
TP	0.85	0.52	0.49	0.013
MicP	0.96	-0.27	0.21	0.220
TN	-0.35	-0.94	0.31	0.097
TC	-0.49	-0.87	-0.77	0.001
pH	-0.65	-0.76	0.18	0.298
Moisture	-0.58	-0.81	0.13	0.395
Floodplain Type			0.47	0.001
Site			0.87	0.001
Topography			0.02	0.780

Table 4.1. Locations and characteristics of the study sites. Soils data were obtained from USDA/NRCS regional soil maps; drainage areas are from the USGS web interface for the National Water Information System (<http://waterdata.usgs.gov>).

Study	Site	River type	Lat/Long	Drainage	Soil Class	†Dominant
2	Nottaway	Alluvial	36°46'13"	3,732	Typic	Cypress, Tupelo
2	Pee Dee	--- " ---	34°12'15"	22,870	Aquic Quartzipsamments	Cypress, Tupelo
2	Ocmulgee	--- " ---	31°55'12"	13,416	Typic Paleudults	Cypress,
1, 2	Wateree	--- " ---	33°49'42"	14,478	Fluvaquentic Dystrochrepts	Cypress, River
1, 2	Black River,	Blackwater	34°45'18"	1,751	Typic Fluvaquent	Sweetgum,
2	Drowning	--- " ---	35°03'40"	474	Fluvaquentic	River Birch,
2	Salkehatchie	--- " ---	32°59'20"	883	Typic Haplohumods/Typic	Tupelo,
2	Satilla River,	--- " ---	31°14'17"	3,108	Fluvaquentic Dystrochrepts	Sycamore, Bald

†The dominant woody vegetation at all eight sites is mixed deciduous bottomland forest dominated by bald cypress (*Taxodium distichum*), sweetgum (*Liquidambar styracifula*), river birch (*Betula nigra*), water tupelo (*Nyssa aquatica*), and willow oak (*Quercus phellos*) (Frederick and Walbridge, unpublished data).

Table 4.2. Oligonucleotide primers for targeting bacterial 16S rDNA and fungal 18S rDNA/ITS genes from total genomic DNA extracted from soils.

Primers	Sequence (5' to 3')	Target gene (bp)	Thermal cycling conditions	Reference
63-F 1387-R	CAG GCC TAA CAC GGG CGG WGT GTA	Bacterial 16S rDNA (1300)	95°C 5 min → 95°C 60 s, 55°C 60 s, 72°C 90 s × 35 cycles → 72°C 10 min	(Marchesi et al. 2001)
*341-F 534-R	CCT ACG GGA GGC ATT ACC GCG GCT GCT	--- " --- (200)		(Muyzer et al. 1993)
*ITS1-F ITS4-R	CTT GGT CAT TTA GAG TCC TCC GCT TAT TGA	Fungal 18S rDNA- ITS (800)	94°C 5 min → 94°C 30 s, 54°C 30 s, 72°C 30 s × 35 cycles → 72°C 10 min	(White et al. 1990, Gardes and Bruns 1993)
ITS2-R	GCT GCG TTC TTC ATC	--- " --- (300)		

*DGGE primers 341-F and ITS1-F were synthesized with a 5' end 40 bp GC clamp to stabilize the melting behavior of the DNA fragments. The GC clamp CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G was added to the bacterial primer 341-F, and CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G was added to the fungal primer ITS1-F.

Table 4.3. (a) Chemical analysis of soils sampled in ridge (RD), dry swale (SWD), and/or flooded swale (SWF) microsite elevations in one alluvial (AL) and one blackwater (BW) FF; and (b) Results of a 2-way ANOVA for the effects of floodplain type (AL, BW), ridge (RD) or swale (SW) microsite elevations, and the interaction term, on soil chemical characteristics. When necessary, data were log transformed to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

Table 4.3 a: Chemical analysis of FF soils.

BW Floodplain	Topo.	TP	MicP	TN	TC	pH	Moisture
Black R., NC	RD 1	5.1	0.17	27.9	437.4	5.2	28.3
--- " ---	RD 2	4.6	0.09	37.5	458.1	5.3	32.1
--- " ---	RD 3	4.6	0.10	80.2	952.1	5.7	29.6
--- " ---	RD 4	3.7	0.01	20.6	326.4	5.5	25.1
--- " ---	RD 5	4.5	0.03	17.1	238.9	5.5	19.3
--- " ---	RD 6	3.7	0.14	19.1	335.8	5.2	19.2
BW-RD Mean (SE)		4.4	0.09	33.7	458.1	5.4	25.6
Black R., NC	SW 1	3.7	0.17	60.5	408.3	5.2	36.6
--- " ---	SW 2	4.7	0.06	74.4	897.8	5.2	30.4
--- " ---	SWF 3	3.5	0.11	49.1	917.2	5.8	83.9
--- " ---	SWF 4	3.6	0.04	71.2	845.5	5.7	84.3
--- " ---	SWF 5	4.9	0.10	61.1	820.7	5.4	79.8
--- " ---	SWF 6	6.5	0.48	40.1	515.6	5.6	69.3
BW-SW Mean (SE)		4.5	0.2	59.4	734.2	5.5	64.1
BW – Overall Mean (SE)		4.4	0.1	46.6	596.2	5.4	44.8
AL Floodplain							
Wateree R., SC	RD 1	12.7	0.13	21.1	298.7	5.2	29.8
--- " ---	RD 2	11.2	0.22	20.0	177.6	5.3	29.3
--- " ---	RD 3	10.7	0.04	44.9	155.9	6.1	26.1
--- " ---	RD 4	11.8	0.10	22.1	222.7	5.8	27.8
--- " ---	RD 5	13.5	0.08	47.7	283.3	5.7	30.1
--- " ---	RD 6	12.9	0.05	23.5	305.1	6.0	27.9
AL-RD Mean (SE)		12.1	0.1	29.9	240.6	5.7	28.5
Wateree R., SC	SW 1	10.6	0.09	47.4	260.1	5.1	28.7
--- " ---	SW 2	11.0	0.11	43.5	371.4	5.8	32.6
--- " ---	SW 3	9.5	0.10	49.0	280.4	5.7	29.0
--- " ---	SW 4	11.2	0.14	22.8	225.7	5.9	27.5
--- " ---	SW 5	12.6	0.25	25.1	290.3	5.2	29.4
--- " ---	SW 6	11.7	0.11	25.1	263.8	5.8	27.2
AL-SW Mean (SE)		11.1	0.1	35.5	281.9	5.6	29.1
AL – Overall Mean (SE)		11.6	0.1	32.7	261.2	5.6	28.8
AL & BW – RD Mean (SE)		8.2	0.1	31.8	349.3	5.5	27.1
AL & BW – SW Mean (SE)		7.8	0.1	47.4	508.1	5.5	46.6

Table 4.3 b: Results of the 2-way ANOVA

	d. f.		TP	MicP	TN	TC	pH	Moisture
Floodplain type, F	1	F	245.4013	0.3579	2.8958	30.0095	2.8735	9.8291
		<i>P</i>	< 0.0001	<i>0.5564</i>	<i>0.1043</i>	< 0.0001	<i>0.1056</i>	0.0052
Topography, T	1	F	0.3804	2.1006	7.2033	6.6798	0.0023	14.5393
		<i>P</i>	<i>0.5444</i>	<i>0.1627</i>	0.0143	0.0177	<i>0.9625</i>	0.0011
F × T	1	F	0.6428	0.0989	2.3073	1.5981	0.7242	13.7190
		<i>P</i>	<i>0.4321</i>	<i>0.7564</i>	<i>0.1444</i>	<i>0.2207</i>	<i>0.4049</i>	0.0014

Table 4.4. (a) Chemical analysis of soils sampled in ridge (RD), dry swale (SWD), and/or flooded swale (SWF) microsite elevations in soils collected from four alluvial (WT: Wateree R., SC; PD: Pee Dee R., SC; NT: Nottaway R., VA; and OC: Ocmulgee R., GA) and four blackwater (BL: Black R., NC; DC: Drowning Cr., NC; ST: Satilla R., GA; and SK: Salkehatchie R., SC) FFs; and (b) Results of the 2-way ANOVA of the effects of floodplain type (AL and BW), microsite elevation (ridge (RD) or swale (SW)), and the interaction term, on soil chemical characteristics. When necessary, data were log transformed to meet the assumption of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

Table 4.4 a: Chemical analysis of FF soils.

BW Floodplain	Topo.	TP	MicP	TN	TC	pH	Moisture
Black R., NC	RD	4.6	0.10	80.2	952.1	5.7	29.6
--- " ---	SWF	3.5	0.11	49.1	917.2	5.8	83.9
Drowning Creek, NC	SWF a	2.7	0.01	33.3	834.3	5.5	59.4
--- " ---	SWF b	2.3	0.04	41.4	768.8	5.5	67.3
Satilla River, GA	RD	1.6	0.12	31.0	552.1	5.6	16.9
--- " ---	SWF	4.2	0.06	56.4	873.7	5.6	28.4
Salkehatchie River, SC	SWF a	9.3	0.08	47.5	792.9	6.5	54.4
--- " ---	SWF b	6.9	0.17	62.5	800.3	6.9	40.3
BW-RD Mean (SE)		3.1	0.1	55.6	752.1	5.6	23.2
BW-SW Mean (SE)		4.8	0.08	48.4	831.2	5.9	55.6
<i>BW – Overall Mean (SE)</i>		4.4	0.09	50.2	811.4	5.9	47.5
AL Floodplain							
Wateree R., SC	RD	13.5	0.08	47.7	283.3	5.7	30.1
--- " ---	SW	12.6	0.25	25.1	290.3	5.2	29.4
Pee Dee River, SC	RD	4.4	0.02	6.4	149.8	5.6	21.1
--- " ---	SWF	5.5	0.01	17.1	321.5	5.4	37.5
Nottaway River, VA	RD	8.5	0.18	60.2	433.1	5.7	36.4
--- " ---	SWF	10.1	0.20	44.9	533.7	5.9	40.6
Ocmulgee River, Ga	RD	6.3	0.01	15.7	153.4	5.9	6.4
--- " ---	SWF	8.9	0.10	26.3	222.4	5.4	32.2
AL-RD Mean (SE)		8.2	0.07	32.5	254.9	5.7	23.5
AL-SW Mean (SE)		9.3	0.1	28.3	342.0	5.5	34.9
<i>AL – Overall Mean (SE)</i>		8.7	0.1	30.4	298.4	5.6	29.2
AL & BW – RD Mean (SE)		6.5	0.09	40.2	420.6	5.7	23.4
AL & BW – SW Mean (SE)		6.6	0.1	40.3	635.5	5.8	47.3

Table 4.4 b: Results of the 2-way ANOVA

	d. f.		TP	MicP	TN	TC	pH	Moisture
Floodplain type, F	1	F	6.0085	0.0710	3.1708	19.9537	0.8284	1.1960
		P	0.0473	0.7980	0.1176	0.0036	0.3962	0.3105
Site [F]		F	5.5589	3.8662	1.9625	5.3933	7.2777	2.2243
		P	0.0278	0.0622	0.2162	0.0299	0.0146	0.1768
Topography, T	1	F	2.0506	0.4486	0.1431	5.7279	0.2843	9.7771
		P	0.2021	0.5279	0.7182	0.0538	0.6131	0.0204
F × T	1	F	0.1986	2.2765	0.0050	0.3229	1.4990	2.3090
		P	0.6715	0.1821	0.9462	0.5905	0.2667	0.1794

Table 4.5. Similarities of dominant **(a)** bacterial 16S rDNA: and **(b)** fungal ITS rDNA, sequences from excised DGGE bands, to clone library sequences (**Appendix Table 4.1**) and their closes relatives to sequences retrieved from GenBank, of soils collected from one alluvial (AL) (Wateree R., SC) and one blackwater (BW) (Black R., NC) FF (**Figures 4.3, 4.4**).

Table 4.5a. Dominant bacterial 16S rDNA sequences from excised DGGE bands.

Band	OTU	Access. #	Score % Sim.	Phylum	Class	Order	Family	Genus	BL	WT	Total
1	31-TB-46	FN433469	850/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocapsa	2		2
2	18-TB-371	FJ529710	784/94	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Actinomadura		4	4
3	11-TB-209	JF510471	1459/90	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	Aciditerrimonas	7		7
4	42-TB-157	EF219138	636/87	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
5	48-TB-186	AF498727	1424/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
6	7-TB-245	AF409023	1629/93	Proteobacteria	Gammaproteobacteria	Chromatiales			2	6	8
7	29-TB-204	HM748714	1596/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1	2	3
8	5-TB-253	AB531426	1764/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	3	10	13
9	4-TB-212	HM748695	1731/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		11	5	16
10	13-TB-243	AY234636	1757/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		6		6
11	26-TB-289	AF498753	1519/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			3	3
12	17-TB-40	AM503077	985/99	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	4		4
13	14-TB-273	AY234650	1275/92	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	5	1	6
14	2-TB-188	HM748653	1461/95	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae	Solibacter	13	7	20
15	15-TB-453	HM748732	942/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			5	5
16	20-TB-439	GU187030	652/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			4	4
17	3-TB-27	HM748709	885/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		12	4	16
18	49-TB-265	AY234650	1356/89	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		2		2
19	16-TB-33	NR-025582	713/91	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		2	2	4
20	12-TB-349	FJ459988	813/97	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		4	3	7
21	6-TB-237	AY234592	1688/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		7	3	10
22	9-TB-100	EF074997	870/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3	4	7
23	10-TB-295	AF498727	1458/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		6	1	7
24	1-TB-463	GQ369128	931/98	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	11	21	32
25	24-TB-280	HM748732	1245/88	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	1	3

26	8-TB-71	AF498722	806/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylocystis	7		7
27	32-TB-264	FJ469913	1546/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2

Table 4.5b. Dominant fungal ITS rDNA sequences from excised DGGE bands.

Band	OTU	Access. #	Score % Sim.	Phylum	Subphylum	Class	Order	Family	Genus	BL	WT	Total
1	19-TF-198	FJ554185	826/93	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae		3	1	4
2	10-TF-61	JN943015	1062/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	7		7
3	20-TF-263	GU934592	1053/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales	Corticaceae	Phanerochaete		3	3
4	3-TF-22	EU598156	1037/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	10	2	12
5	6-TF-286	EU784211	909/95	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Entolomataceae	Entoloma		8	8
6	2-TF-213	JF439485	1138/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	3	15	18
7	11-TF-222	AB176571	1081/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales		Daedaleopsis		6	6
8	30-TF-320	GU479272	928/94	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Hymenogastraceae	Hymenogaster		3	3
9	16-TF-109	EU598162	983/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	5		5
10	5-TF-216	AY312985	1088,98	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Hebeloma		9	9
11	12-TF-232	AY656948	870/92	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae			6	6
12	15-TF-141	JF834358	896/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	5		5
13	17-TF-175	FJ554007	965/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	0	4	4
14	14-TF-254	AJ633598	1050/97	Basidiomycota							5	5
15	23-TF-159	HQ714730	939/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	3		3
16	7-TF-37	EU598194	752/91	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	6	2	8
17	9-TF-211	EU819478	1116/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria	3	5	8
18	27-TF-318	EU819493	1140/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula		3	3
19	1-TF-67	FN428924	926/100	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales		Cryptococcus	18	8	26
20	29-TF-311	EU167594	966/99	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella		3	3
21	24-TF-206	HM488478	902 98	Ascomycota	Pezizomycotina	Leotiomycetes				1	2	3
22	13-TF-123	HQ157874	750/92	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae		5		5
23	25-TF-75	HQ630960	1051/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	1	2	3
24	4-TF-127	DQ777988	1051/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	9	1	10
25	18-TF-225	JF908544	961/95	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Pezizaceae	Peziza		4	4

26	46-TF-19	EU598153	992/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
27	26-TF-315	HM176560	1079/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	2	1	3
28	32-TF-96	JF735304	987/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Ilyonectria	2		2
29	22-TF-3	U57496	715/91	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae	Arachnopeziza	3		3
30	123-TF-310	FJ612884	684/91	Ascomycota	Pezizomycotina	Sordariomycetes					1	1
31	21-TF-134	EU819480	957/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	3		3
32	38-TF-100	EF694630	968/99	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	2		2
33	31-TF-199	HM189876	893/98	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales			1	1	2
34	8-TF-381	AJ557830	1059/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	6	2	8

Table 4.6. Similarities of dominant **(a)** bacterial 16S rDNA; and **(b)** fungal ITS rDNA, sequences from excised DGGE bands, to clone library sequences (**Appendix Table 4.2**) and their closes relatives, to sequences retrieved from GenBank, of soils collected from four alluvial (WT: Wateree R., SC; PD: Pee Dee R., SC; NT: Nottaway R., VA; and OC: Ocmulgee R., GA) and four blackwater (BL: Black R., NC; DC: Drowning Cr., NC; ST: Satilla R., GA; and SK: Salkehatchie R., SC) FFs (**Figures 4.5, 4.6**).

Table 4.6a. Dominant bacterial 16S rDNA sequences from excised DGGE bands.

Band	OTU	Accession #	Score %Sim.	Phylum	Class	Order	Family	Genus	Ridge	Swale	Total
1	10-MB-440	AF498727	1672/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	3	5
2	20-MB-68	HM748754	1709/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3		3
3	30-MB-59	AF482687	1587/92	Proteobacteria	Deltaproteobacteria	Myxococcales			2		2
4	15-MB-641	D16209	1387/91	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Rhodocyclus	3		3
5	11-MB-627	NR-043898	1764/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Edaphobacter	3	1	4
6	4-MB-385	AF409023	1807/95	Proteobacteria	Gammaproteobacteria	Chromatiales			2	6	8
7	1-MB-559	AB531426	1997/98	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	6	11	17
8	36-MB-381	AY234546	1620/96	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae			2	2
9	7-MB-164	AY234650	1506/91	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		4	2	6
10	2-MB-367	HM748695	1783/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		4	8	12
11	12-MB-350	AY234636	1978/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	2	4
12	19-MB-441	GU187030	1526/91	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae		1	2	3
13	22-MB-86	HM748653	1681/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		2		2
14	9-MB-443	AF498753	1923/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3	2	5
15	43-MB-496	AM503077	1615/98	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1	1	2
16	16-MB-453	AB250621	1657/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodomicrobium		3	3
17	6-MB-513	HM748653	1993/96	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae	Solibacter	4	2	6
18	13-MB-506	HM748732	1770/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	1	3
19	42-MB-469	AY234546	1701/94	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae			2	2
20	53-MB-389	GU187030	1229/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
21	8-MB-32	EU938323	1893/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	3	2	5
22	3-MB-383	HM748732	1714/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3	5	8
23	17-MB-392	AF498727	1472/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	2	3
24	5-MB-526	GQ369128	1760/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	3	4	7
25	18-MB-210	AY234475	1456/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3		3

26	32-MB-455	HM748709	1851/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		1	1	2
27	21-MB-196	HM748695	1814/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
28	39-MB-359	HM748732	1698/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	1	2
29	38-MB-522	AB681929	1842/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1	1	2
30	95-MB-78	AY234650	1461/90	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1

Table 4.6b. Dominant fungal ITS rDNA sequences from excised DGGE bands.

Band	OTU	Accession	Score % Sim.	Phylum	Subphylum	Class	Order	Family	Genus	RD	SW	Total
1	14-MF-18	EU186277	1266/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Tricholoma	4		4
2	3-MF-266	JF439485	1181/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	5	7	12
3	103-MF-351	AY312985	1140/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Hebeloma		1	1
4	48-MF-16	FJ554007	987/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	1		1
5	11-MF-397	HQ873381	425/88	Zygomycota						1	3	4
6	6-MF-314	DQ195594	763/86	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae			6	6
7	27-MF-145	AJ633598	1064/97	Basidiomycota						2		2
8	21-MF-373	JF908140	638/84	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Inocybaceae	Inocybe		3	3
9	15-MF-229	EU819478	1267/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria		4	4
10	7-MF-49	EU819493	1120/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Amanitaceae	Amanita	6		6
11	2-MF-271	FN428924	935/99	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales		Cryptococcus	18	12	30
12	25-MF-441	JN882326	364/81	Chytridiomycota		Monoblepharidomycetes	Monoblepharidales	Gonapodyaceae	Gonapodya	2		2
13	9-MF-96	FJ845443	678/85	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Tricholoma	1	4	5
14	105-MF-372	HM488478	872/96	Ascomycota	Pezizomycotina	Leotiomyces					1	1
15	38-MF-435	HQ157874	763/92	Ascomycota	Pezizomycotina	Leotiomyces	Helotiales	Helotiaceae		1	1	2
16	22-MF-359	HQ630960	1131/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	3		3
17	18-MF-127	DQ777988	1192/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	3		3
18	33-MF-220	FN397177	492/84	Ascomycota							2	2
19	8-MF-26	EU598153	1140/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	2	3	5
20	4-MF-159	HM176576	1101/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	8		8
21	12-MF-23	AM084698	928/99	Ascomycota	Pezizomycotina	Dothideomycetes			Cenococcum	4	1	5
22	32-MF-217	JF735304	989/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Ilyonectria	2		2
23	17-MF-391	GU566216	1016/98	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium		4	4
24	10-MF-383	HM189876	985/92	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales			3	2	5
25	1-MF-346	AJ557830	1075/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	36	97	133

26	13-MF-70	EU557361	950/99	Ascomycota	Pezizomycotina	Sordariomycetes	Microascales	Halosphaeriaceae	Sigmoidea	4		4
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Table 4.7. Shannon diversity index (H), richness (S), and evenness (E), of bacterial and fungal DGGE fingerprint profiles, in soils from ridge (RD), dry swale (SWD), and/or flooded swale (SWF) microsite elevations, from one alluvial (AL) and one blackwater (BW) FF.

		Bacterial DGGE profile			Fungal DGGE profile		
BW Floodplain	Topo.	<i>H</i>	<i>S</i>	<i>E_H</i>	<i>H</i>	<i>S</i>	<i>E_H</i>
Black R., NC	RD 1	2.25	14	0.85	1.77	9	0.81
--- " ---	RD 2	2.12	14	0.80	1.22	5	0.76
--- " ---	RD 3	2.01	14	0.76	1.77	7	0.91
--- " ---	RD 4	2.36	19	0.80	1.40	5	0.87
--- " ---	RD 5	2.45	17	0.86	2.25	11	0.94
--- " ---	RD 6	2.51	19	0.85	1.29	5	0.80
Black R., NC	SW 1	1.56	13	0.61	1.60	7	0.82
--- " ---	SW 2	2.31	13	0.90	1.75	7	0.90
--- " ---	SWF 3	2.37	17	0.84	2.48	13	0.96
--- " ---	SWF 4	2.05	17	0.72	1.54	6	0.86
--- " ---	SWF 5	1.89	11	0.79	1.30	4	0.93
--- " ---	SWF 6	2.25	13	0.88	1.50	5	0.93
<i>BW – Overall Mean</i>		<i>2.18</i>	<i>15</i>	<i>0.81</i>	<i>*1.66^B</i>	<i>**7^B</i>	<i>0.87</i>
AL Floodplain							
Wateree R., SC	RD 1	2.05	13	0.80	2.22	10	0.96
--- " ---	RD 2	2.23	15	0.83	2.25	13	0.88
--- " ---	RD 3	2.03	13	0.79	2.02	8	0.97
--- " ---	RD 4	2.00	13	0.78	1.71	8	0.82
--- " ---	RD 5	2.27	15	0.84	2.38	14	0.90
--- " ---	RD 6	2.19	14	0.83	1.57	7	0.81
Wateree R., SC	SW 1	2.42	17	0.85	1.69	7	0.87
--- " ---	SW 2	2.41	16	0.87	2.28	13	0.89
--- " ---	SW 3	2.28	16	0.82	2.08	10	0.90
--- " ---	SW 4	2.45	16	0.88	2.11	9	0.96
--- " ---	SW 5	2.23	14	0.84	2.15	10	0.93
--- " ---	SW 6	2.23	16	0.81	2.18	11	0.91
<i>AL – Overall Mean</i>		<i>2.23</i>	<i>15</i>	<i>0.83</i>	<i>*2.05^A</i>	<i>**10^A</i>	<i>0.90</i>
AL & BW – RD Mean		2.21	15	0.82	1.82	9	0.87
AL & BW – SW Mean		2.20	15	0.82	1.89	9	0.91

* $p = 0.007$, $F = 8.880$, ** $p = 0.009$, $F = 8.250$

Table 4.8. Shannon diversity index (H), richness (S), and evenness (E), of bacterial and fungal DGGE fingerprint profiles of soils from ridge (RD), dry swale (SWD), and/or flooded swale (SWF) microsites in four alluvial (AL) and four blackwater (BW) FFs.

		Bacterial DGGE profile			Fungal DGGE profile		
BW Floodplain	Topo.	<i>H</i>	<i>S</i>	<i>E_H</i>	<i>H</i>	<i>S</i>	<i>E_H</i>
Black R., NC	RD	2.44	22	0.79	1.60	6	0.89
--- " ---	SWF	2.82	22	0.91	2.54	15	0.94
Drowning Creek, NC	SWF a	2.60	19	0.88	0.70	4	0.50
--- " ---	SWF b	2.75	21	0.90	1.00	5	0.62
Satilla River, GA	RD	2.51	16	0.90	1.69	7	0.87
--- " ---	SWF	2.19	15	0.81	1.79	13	0.70
Salkehatchie River, SC	SWF a	2.45	14	0.93	1.32	8	0.63
--- " ---	SWF b	2.49	14	0.94	1.69	10	0.74
<i>BW – Overall Mean</i>		<i>2.53</i>	<i>18</i>	<i>0.88</i>	<i>1.54</i>	<i>9</i>	<i>0.74</i>
AL Floodplain							
Wateree R., SC	RD	2.81	19	0.95	2.27	13	0.89
--- " ---	SW	2.66	17	0.94	1.11	6	0.62
Pee Dee River, SC	RD	2.73	18	0.95	1.90	8	0.92
--- " ---	SWF	2.44	17	0.86	1.63	7	0.84
Nottaway River, VA	RD	2.76	19	0.94	1.44	7	0.74
--- " ---	SWF	2.71	21	0.89	1.95	11	0.82
Ocmulgee River, Ga	RD	2.64	19	0.90	1.59	6	0.89
--- " ---	SWF	2.91	22	0.94	1.16	5	0.72
<i>AL – Overall Mean</i>		<i>2.71</i>	<i>19</i>	<i>0.92</i>	<i>1.63</i>	<i>8</i>	<i>0.80</i>
AL & BW – RD Mean		2.65	19	0.90	1.75	8	0.87
AL & BW – SW Mean		2.60	18	0.90	1.49	8	0.71

CHAPTER 5

General Conclusions

Although the vulnerability of natural waters to anthropogenic phosphorus (P) enrichment has long been recognized, successful remediation strategies to provide a sound basis for the management of aquatic ecosystems are much needed (Fisher et al. 1995, Costanza et al. 1997). The nutrient retention and transformation capacities of riparian wetlands (including floodplain forests (FFs)) may be one mode of defense against reducing water-associated contaminants and excess nutrients (such as P), to achieve the required P load reduction in riverine systems, and mitigate the consequent eutrophication of aquatic ecosystems. The goal of this dissertation was to improve our understanding of the dominant mechanisms of P retention and transformation in floodplain forests of the southeastern (SE) US.

To explore these processes of P retention and transformation during flooding events, I characterized the various forms and relative proportions of inorganic and organic P chemistries (using ^{31}P nuclear magnetic resonance (NMR) spectroscopy) in inflow and outflow floodwaters in two Alluvial (AL) and two Blackwater (BW) FFs (**Chapter 2**), and assessed the dominant fate(s) of inorganic P received in floodwaters by adding $^{32}\text{PO}_4^{3-}$ to soil (collected at 2 AL and 2 BW FFs) mesocosms during simulated flooding events in the laboratory (**Chapter 3**). Despite the significant role of microorganisms in soil P dynamics, little is known about the metabolic processes or taxonomic composition of the bacteria and fungi involved in the retention and transformation of P in FFs. To approach this poorly explored subject, I examined variations in bacterial and/or fungal community structure as a function of floodplain type (4 AL vs. 4 BW), microsite elevation (high elevation ridge, RD vs. low elevation SW), and associated variations in

soil chemical parameters (total carbon (C), nitrogen (N), P, microbial P, soil pH and moisture content) in FFs, and how these variations, in turn, might affect the retention and transformation of P (**Chapter 4**).

A number of studies have examined rates of P retention and transformation in FFs (Mitsch et al. 1979, Brinson et al. 1984, Craft and Casey 2000, Stoeckel and Miller-Goodman 2001, Bruland and Richardson 2005, Noe and Hupp 2007), and yet none have detailed the various forms and concentrations of P, particularly organic P, in inflow versus outflow floodwaters during overbank flooding. We developed an improved method in the use of ^{31}P NMR spectroscopy to characterize both dissolved and particulate forms of inorganic and organic P in freshwaters (Cade-Menun et al. 2006), which facilitates these types of analyses in floodwaters entering FFs in the SE US (Chapter 2). Total P (the sum of dissolved P (DP) and particulate P (PP)) concentrations were similar in floodwaters collected at both the river inlet (RI) and within the floodplain (FP) at AL and BW floodplain types, which suggests that TP pools were neither an overall sink nor source from AL and BW floodplains. But rather an indication of the transformation of P (from inorganic (P_i) to organic (P_o) form) carried with floodwaters prior to exiting the FF. During these spring flooding events, AL and BW floodplains transformed 21 and 11 % of the sum of NMR-visible dissolved inorganic P (DIP) forms, respectively, and of these DIP forms, the most readily bioavailable – orthophosphate in river waters was transformed at rates of 26 and 17 % within AL and BW floodplains, respectively. While total dissolved P (TDP) and dissolved orthophosphate concentrations declined from RI to FP, total dissolved organic P (DOP) and dissolved pyrophosphate concentrations were significantly higher in FP than in RI waters. Similarly, orthophosphate monoesters (comprising the majority of the DOP

pool) were significantly higher in FP than in RI floodwaters in both AL and BW systems. This conversion of DIP forms (e.g. orthophosphate) to dissolved condensed inorganic P forms (pyrophosphates) and low molecular weight DOP compounds (e.g. orthophosphate monoesters) suggests biological P transformation, an important process occurring during flooding events likely leads to less available P forms being carried with floodwaters exiting these FFs, thus reducing their potential to cause the eutrophication of downstream waters.

To assess the dominant fate(s) of inorganic P received in floodwaters, I determined the distribution of P in soils (by a detailed sequential soil P fractionation procedure) after adding $^{32}\text{PO}_4^{3-}$ to soil mesocosms during simulated flooding events in the laboratory (**Chapter 3**). While a majority of the $^{32}\text{PO}_4^{3-}$ introduced in floodwaters was transformed to organic P forms ($^{32}\text{P}_o$) in the river water column in BW rivers, a substantial amount of added $^{32}\text{PO}_4^{3-}$ was additionally transformed to $^{32}\text{P}_o$ in headwaters upon contact with the soils surface in AL and BW cores. This rapid and substantial transformation of P suggests biotic uptake of $^{32}\text{PO}_4^{3-}$ in floodwaters. Floodplain soils had superior P retention capacities, retaining an average 88 % of ^{32}P label introduced with floodwaters that was distributed within the soil fractions (73 %), microbial biomass, (9 %) and associated with the root/rhizosphere (6 %) across AL and BW soil cores. Most of the ^{32}P retained was associated with the NaOH, BD, Res, and HA extractable soil fractions, sorbed to aluminum (Al) and/or clay minerals in the NaOH, Res, and H_2O extractable fractions, or strongly associated with iron (Fe) in the BD and HCl fractions in both AL and BW soils. The ^{32}P extracted from the HA-P fraction, however, differed between AL and BW soils; the accumulation of ^{32}P in the HA fraction in BW soils was more strongly linked to the presence of Al, whereas the accumulation of ^{32}P in the HA fraction in AL soils was more strongly linked

to the presence of Fe. The fact that concentrations of HA-Fe in AL soils were not significantly reduced with prolonged flooding (i.e., over the 8 day experimental period), suggests that they may represent stable Fe⁺² humic compounds.

Results also suggest that floodplain soils act as a second line of defense protecting water quality, by retaining P inputs that are not initially transformed within the water column (river- and headwaters) during flooding events. Although a majority of the ³²P added to soils was recovered in the plant available (NaOH, BD, and HA) and occluded-P (Res) fractions, the highest specific activities (ratio of ³²P activity/P concentration) were observed in biologically dominant and available P pools (H₂O extractable fraction, root/rhizosphere and microbial biomass pools), suggesting the importance of these pools in removing and/or transforming P_i inputs received in floodwaters. In combination, these processes reduce P_i inputs to aquatic systems downstream.

Despite our knowledge of the significant role of microorganisms in wetland (including FF) soil P dynamics (Richardson and Marshall 1986, Walbridge 1991, Bedford et al. 1999, Wright et al. 2001, Hogan et al. 2004), little is known about the metabolic processes or taxonomic composition of the bacteria and fungi involved in the retention and transformation of P in wetlands, especially FFs. In **Chapter 4**, I compared in detail soil microbial community composition (using a protocol combining bacterial-16S-rDNA and fungal-18S/ITS-rDNA cloning with denaturing gradient gel electrophoresis, DGGE) as a function of microtopographic elevation and FF type in SE US, as related to soil chemical parameters (i.e., pH, C, N, P, and microbial biomass P), to examine their effects on microbial community structure. Jaccards

similarity assessment of DGGE profiles divided soils into two clusters that corresponded with differences in floodplain type (AL vs. BW). In both the clone library and DGGE fingerprint data, the dominant bacterial taxa belonged to the *Acidobacteria* and *Alphaproteobacteria* groups, and the most prevalent fungal taxa belonged to the *Ascomycota* and *Basidiomycota* subdivisions. Detrended correspondence analysis of DGGE fingerprint data and the subsequent fitting of soil chemical parameters to ordination plots, were in close agreement with the results obtained by cluster analysis, and revealed that soil P and pH were associated with the composition of bacterial and fungal communities in AL FFs, whereas, soil C was strongly correlated with the microbial community profiles in BW FFs. These results provide taxonomic identification of the dominant bacteria and fungi in FF soils, and show that differences in floodplain type (AL vs. BW systems of the SE US) are associated with differences in soil C, P and pH, that are important predictors of microbial composition.

The central goal of the research presented in this dissertation was to improve our understanding of the dominant mechanisms of P retention and transformation in FFs of the southeastern (SE) US. To accomplish this goal I used state-of-the-art technologies and novel approaches to examine P retention and transformation capacities in these unique wetlands. My findings clearly indicate that these floodplain wetlands are indeed valuable ecosystems that have profound effects on the biogeochemical retention and transformation of excess P loadings carried with floodwaters, playing an important role in maintaining water quality in downstream aquatic ecosystems. It is forecasted that by 2025 as much as two-thirds (an estimated 5 out of the 8 billion people) of the world population will experience severe water scarcity due to climate change impacts and poor water quality resulting from the eutrophication of potable freshwater

reservoirs (Bennett et al. 2001, Arnell 2004, Conley et al. 2009, Erwin 2009, Hoekstra et al. 2012). North America is likely to be impacted by these trends (Arnell 2004, Conley et al. 2009, Hoekstra et al. 2012), and as such future policy decisions may be dependent on the prioritization of riparian wetlands (particularly FFs) based on their ecosystems functions and values. Yet, wetlands in the US are lost at an alarming rate, (Kress et al. 1996, Mitsch and Gosselink 2007), and freshwater marshes and forested wetlands are the hardest hit, with loss rates of 7 and 5 % per decade, respectively. Despite their importance, in the southern US alone, bottomland hardwood forests (including FFs) continue to be lost at a rapid rate, having declined by 6.5 million ha (77 % loss) between 1883 - 1991 (Mitsch and Gosselink 2007). In fact, three of the ten FFs studied in this dissertation have been destroyed in the past four years, by draining and clearing forests for timber (Nottaway River, VA), agriculture (Black River, NC), or conversion to pine plantation forestry (Wateree River, SC). This destruction of FF wetlands and their associated biogeochemical function is likely to add substantially to the eutrophication problem (Carpenter 2005, Mitsch and Gosselink 2007, Dodds et al. 2009), and will continue to do so unless measures are taken to assess the sustainability of the ecosystem services they provide, and successfully replicate them through conservation, creation, and/or restoration of these wetland types.

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APPENDICES

CHAPTER 2:

Appendix Table 2.1: Results of a 2-way ANOVA for floodplain type (AL and BW) and sampling location (RI and FP) (and interaction term) in: a) dissolved and particulate Total P, MRP, C, Al, Fe, pH, and conductivity measured by chemical analysis, b) NMR visible dissolved P forms, and c) NMR visible particulate P forms. Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold. Abbreviations: TDP, total dissolved P; MRP, molybdate reactive P; C, carbon; Al, aluminum; Fe, iron; Cond, conductivity; TPP, total particulate P; orthoP, orthophosphate; pyroP, pyrophosphate; polyP, polyphosphate; PA, phytate; *scyllo*-IP, *scyllo*-inositol phosphate; GP, glycerophosphate; DNA, deoxyribonucleic acid; phon, phosphonate.

Table 2.1 a: Dissolved and particulate Total P, MRP, C, Al, Fe, as well as pH and conductivity.

Measured Components		Dissolved									
	d. f.	TDP		SRP		C		Al		Fe	
		F	P	F	P	F	P	F	P	F	P
Floodplain type, F	1	1.896	0.218	0.117	0.744	9.856	0.020	12.787	0.012	11.676	0.014
RI/FP location, L	1	2.794	0.146	4.796	0.071	0.054	0.823	13.602	0.010	0.429	0.537
F × L	1	15.912	0.007	0.214	0.660	1.834	0.224	13.735	0.010	0.183	0.684
Site [F]	6	20.205	0.001	6.820	0.017	0.951	0.524	7.353	0.014	2.697	0.126
		Dissolved <i>cont.</i>				Particulate					
	d. f.	pH		Cond.		TPP		C			
		F	P	F	P	F	P	F	P		
Floodplain type, F	1	24.272	0.003	0.107	0.754	2.044	0.203	1.342	0.300		
RI/FP location, L	1	1.116	0.331	0.621	0.461	0.001	0.973	0.666	0.452		
F × L	1	1.415	0.280	0.033	0.862	4.704	0.073	0.822	0.406		
Site [F]	1	0.6526	0.691	9.208	0.008	19.078	0.001	4.878	0.053		

Table 2.1 b: NMR visible dissolved P forms.

Dissolved P forms		Inorganic P								Orthophosphate monoesters					
	d. f.	orthoP		pyroP		Polyp		$\sum P_i$		PA	<i>scyllo</i> -IP		GP		
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>		
Floodplain type, F	1	0.441	0.531	3.279	0.120	2.695	0.152	0.055	0.822	0.286	0.612	0.808	0.403	0.041	0.846
RI/FP location, L	1	10.145	0.019	9.996	0.020	2.695	0.152	6.449	0.044	18.127	0.005	1.468	0.271	3.496	0.111
F × L	1	0.854	0.391	0.001	0.972	2.695	0.152	0.786	0.409	0.307	0.854	2.675	0.153	0.691	0.438
Site [F]	6	5.333	0.031	3.836	0.063	1.000	0.500	7.699	0.013	17.309	0.001	0.890	0.554	6.558	0.019
		Orthophosphate monoesters cont.				Orthophosphate diesters									
	d. f.	Other mono		$\sum$ mono		DNA		other di		$\sum$ di	phon		$\sum P_o$		
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>		
Floodplain type, F	1	0.001	0.975	0.041	0.847	0.022	0.887	0.258	0.629	0.118	0.742	1.000	0.356	0.055	0.822
RI/FP location, L	1	1.968	0.210	5.831	0.048	3.107	0.128	0.039	0.850	2.315	0.179	1.000	0.356	6.449	0.044
F × L	1	1.216	0.312	0.824	0.399	0.194	0.675	0.135	0.726	0.009	0.926	1.000	0.356	0.786	0.409
Site [F]	6	5.720	0.026	8.159	0.011	2.732	0.123	0.696	0.665	1.958	0.217	1.000	0.500	7.699	0.013

Table 2.1 c: NMR visible particulate P forms.

Particulate P forms		Inorganic P								Orthophosphate monoesters					
	d. f.	orthoP		pyroP		Polyp		ΣP_i		PA		scyllo-IP		GP	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
Floodplain type, F	1	3.924	0.095	0.002	0.960	1.000	0.356	4.843	0.070	0.806	0.404	13.040	0.011	2.129	0.195
RI/FP location, L	1	0.066	0.806	1.600	0.253	1.000	0.356	0.016	0.905	5.128	0.064	1.112	0.332	0.349	0.576
F $\times$ L	1	1.158	0.323	5.627	0.055	1.000	0.356	0.832	0.397	6.000	0.049	1.095	0.336	0.444	0.530
Site [F]	6	6.525	0.019	10.737	0.005	1.000	0.500	5.379	0.030	9.879	0.007	6.437	0.020	4.229	0.051
		Orthophosphate monoesters cont.				Orthophosphate diesters									
	d. f.	other mono		Σ mono		DNA		other di		Σ di		phon		ΣP_o	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
Floodplain type, F	1	1.485	0.269	6.847	0.040	0.994	0.357	1.105	0.334	1.056	0.344	2.979	0.135	4.843	0.070
RI/FP location, L	1	0.164	0.699	0.004	0.950	0.644	0.453	0.489	0.511	0.629	0.458	0.005	0.945	0.016	0.905
F $\times$ L	1	1.735	0.236	0.318	0.593	3.424	0.114	3.880	0.096	3.622	0.106	0.005	0.945	0.832	0.397
Site [F]	6	11.568	0.004	4.111	0.055	9.837	0.007	9.506	0.007	9.684	0.007	0.503	0.788	5.379	0.030

CHAPTER 3:

Appendix Table 3.1: Results of a 2-way ANOVA for floodplain type (AL and BW), incubation period (non-flooded, 40 h, 8 d), and interaction term for total P concentration and ^{32}P activity recovered in soils, headwater, and drainwater (**Table 3.3**). Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

			Head-water		Root		Microbial biomass		Soil fractions		Drain-water	
	d. f.		P ($\mu\text{g l}^{-2}$)	^{32}P (% Recov.)	P (g m^{-2})	^{32}P (% Recov.)	P (g m^{-2})	^{32}P (% Recov.)	P (g m^{-2})	^{32}P (% Recov.)	P ($\mu\text{g l}^{-2}$)	^{32}P (% Recov.)
Floodplain type, F	1	F	3.3251	0.5878	0.1216	1.0993	0.0470	8.2108	1.6732	1.5405	0.0063	0.5641
		P	0.2098	0.5234	0.7606	0.4044	0.8485	0.1033	0.3251	0.3404	0.9439	0.5309
Site [F]	2	F	8.7834	1.9719	2.9534	0.4789	0.3078	0.4543	13.9388	3.5830	0.6637	1.9504
		P	0.1022	0.3365	0.2529	0.6762	0.7502	0.6876	0.0147	0.2182	0.6011	0.3389
Incubation, I	1	F	0.0668	0.0244	0.7023	0.0004	2.4443	1.5480	2.6812	0.1618	0.7603	0.4653
		P	0.8202	0.8902	0.4901	0.9866	0.2025	0.3395	0.1825	0.7264	0.4738	0.5655
I $\times$ F	1	F	0.0054	0.4573	0.1142	0.1005	7.4280	0.0001	0.0240	0.9324	0.8381	0.2048
		P	0.9479	0.5686	0.7675	0.7812	0.0450	0.9939	0.9764	0.4361	0.4552	0.6951
I $\times$ Site [F]	2	F	1.8194	8.2375	2.7887	4.9135	2.1301	3.1413	4.4840	3.1114	9.4145	2.3648
		P	0.1838	0.0019	0.0823	0.0163	0.1035	0.0614	0.0063	0.0629	0.0012	0.1164

Appendix Table 3.2: Results of a 2-way ANOVA for floodplain type (AL and BW), incubation period (control, 40 h, 8 d), and interaction term for total P content (analyzed by Kjeldahl digest) and soil chemical and physical characteristics (**Table 3.4**). Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

			Distribution of P in Soil, Head-water and Drain-water pools				
	d. f.		Total P	C	N	pH	Moisture
Floodplain type, F	1	F	1.4677	0.4249	4.4280	75.1828	0.2057
		P	0.3494	0.5814	0.1700	0.0130	0.6946
Site [F]	2	F	36.3954	5.2152	5.3730	1.3635	8.0484
		P	0.0023	0.0717	0.0656	0.3377	0.0373
Incubation, I	1	F	0.6509	2.6310	4.1750	14.9036	1.1707
		P	0.5692	0.1865	0.1049	0.0140	0.3979
I × F	1	F	0.2388	0.6583	1.9323	2.8341	0.1600
		P	0.7980	0.5660	0.2587	0.1712	0.8573
I × Site [F]	2	F	3.5086	2.1086	1.1435	0.5956	3.6161
		P	0.0329	0.1063	0.3566	0.6687	0.0169

Appendix Table 3.3: Results of a 2-way ANOVA for floodplain type (AL and BW), incubation period (control, 40 h, 8 d), and interaction term for total P content in sequentially extracted fractions (Paludan and Jensen 1995) (**Table 3.5**). Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

P (g m ⁻²)		Distribution in soil fractions						
	d. f.		H ₂ O-P	BD-P	NaOH-P	HA-P	HCl-P	Res-P
Floodplain type, F	1	F	1.9856	0.0000	1.2134	0.9648	0.5969	1.9580
		P	0.2942	0.9961	0.3855	0.4295	0.5206	0.2967
Site [F]	2	F	11.5183	27.9299	8.7954	8.9451	13.0160	19.5393
		P	0.0168	0.0029	0.0332	0.0304	0.0172	0.0071
Incubation, I	1	F	6.1987	10.6092	4.0701	1.4565	5.7828	1.7125
		P	0.0595	0.0252	0.1086	0.3348	0.0660	0.2902
I × F	1	F	1.0773	2.8471	2.2623	0.2977	0.9947	0.1468
		P	0.4224	0.1703	0.7816	0.7577	0.4460	0.8679
I × Site [F]	2	F	1.0578	1.1119	6.8597	2.5082	9.8096	1.9711
		P	0.3957	0.3706	0.0006	0.0645	<0.0001	0.1264

Appendix Table 3.4: Results of a 2-way ANOVA for floodplain type (AL and BW), incubation period (control, 40 h, 8 d), and interaction term for ^{32}P recovered in sequentially extracted fractions (Paludan and Jensen 1995) (**Table 3.6**). Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

^{32}P (% recovery)			Distribution in soil fractions					
	d. f.		$\text{H}_2\text{O}-^{32}\text{P}$	$\text{BD}-^{32}\text{P}$	$\text{NaOH}-^{32}\text{P}$	$\text{HA}-^{32}\text{P}$	$\text{HCl}-^{32}\text{P}$	$\text{Res}-^{32}\text{P}$
Floodplain type, F	1	F	0.2348	0.2154	0.0067	0.0040	0.1406	0.4182
		<i>P</i>	0.6759	0.6882	0.9421	0.9555	0.7437	0.5841
Site [F]	2	F	15.8860	11.5738	16.8031	10.8736	7.1238	1.9834
		<i>P</i>	0.0592	0.0795	0.0562	0.0842	0.1230	0.3352
Incubation, I	1	F	1.0341	0.1939	1.8800	0.6899	1.4797	1.4033
		<i>P</i>	0.4162	0.7027	0.3039	0.4936	0.3479	0.3579
$I \times F$	1	F	3.7131	0.2809	4.3658	0.4603	0.3096	0.2167
		<i>P</i>	0.1938	0.6491	0.1719	0.5674	0.6339	0.6873
$I \times \text{Site [F]}$	2	F	1.7844	1.2830	0.4113	0.5560	3.1260	2.5093
		<i>P</i>	0.1895	0.2955	0.6674	0.5807	0.0622	0.1024

Appendix Table 3.5: Results of a 2-way ANOVA for floodplain type (AL and BW), incubation period (control, 40 h, 8 d), and interaction term for the proportion of organic P in TP measured in headwater, drainwater, microbial biomass, and sequentially extracted soil fractions (**Table 3.7**). Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

			Headwater	Drainwater	Microbial biomass		H ₂ O-P		BD-P	NaOH-P		HCl-P	Soil sum
	d. f.		³² P _o	³² P _o	P _o	³² P _o	P _o	³² P _o	³² P _o	P _o	³² P _o	³² P _o	³² P _o
Floodplain type, F	1	F	0.3004	1.7748	0.0326	0.3581	2.6053	6.3207	3.5260	6.8360	0.4317	0.3416	0.9678
		P	0.6387	0.3139	0.8734	0.6103	0.2479	0.1284	0.2012	0.1204	0.5786	0.1966	0.4289
Site [F]	2	F	10.0308	0.2778	7.5368	0.3162	1.4219	3.2508	29.7833	18.0156	23.0879	0.3870	251.0598
		P	0.0907	0.7826	0.1171	0.7598	0.3301	0.2353	0.0325	0.0091	0.0415	0.7210	0.0040
Incubation, I	1	F	0.6472	3.7426	4.1842	0.5621	0.4665	9.9714	6.4306	2.0199	0.0012	0.5447	9.2996
		P	0.5056	0.1926	0.1774	0.5316	0.6575	0.0873	0.1257	0.2475	0.9757	0.5373	0.0928
I × F	1	F	0.9482	0.5764	4.5154	0.0767	3.6984	19.4218	4.7183	0.7529	1.4074	0.5447	15.7167
		P	0.4329	0.5270	0.1675	0.8078	0.1232	0.0478	0.1610	0.5278	0.3573	0.5373	0.0581
I × Site [F]	2	F	1.7875	3.3399	2.2545	2.7649	0.8752	0.6766	0.4283	3.8693	2.9326	2.9424	0.2021
		P	0.1889	0.0533	0.1267	0.0831	0.4912	0.5178	0.6567	0.0126	0.0725	0.0720	0.8184

Appendix Table 3.6: Results of a 2-way ANOVA for floodplain type (AL and BW), incubation period (control, 40 h, 8 d), and interaction term for Specific Activity ($SA: ^{32}P/P$, $SA_i: ^{32}P_i/P_i$, $SA_o: ^{32}P_o/P_o$) in sequentially extracted fractions (Paludan and Jensen 1995) (**Table 3.8**). Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

Specific Activity	Soil pools and fractions													
	d. f.		Root	Microbial			H ₂ O		BD	NaOH		HA	HCl	Res
			SA	SA	SA _i	SA _o	SA	SA _i	SA	SA	SA _i	SA	SA	SA
Floodplain type, F	1	F	3.0957	2.3845	4.9961	5.8229	2.5597	0.0358	0.6256	1.4912	15.0367	0.9528	0.0023	2.7584
		<i>P</i>	0.2204	0.2625	0.1549	0.1327	0.2508	0.8675	0.5119	0.3465	0.0605	0.4320	0.9661	0.2386
Site [F]	2	F	1.9869	0.7124	0.0540	1.2331	1.2702	1.4610	1.9873	0.7457	0.4340	1.4395	0.4418	1.0454
		<i>P</i>	0.3348	0.5840	0.9488	0.4478	0.4405	0.4063	0.3347	0.5728	0.5777	0.4099	0.6936	0.4889
Incubation, I	1	F	0.6100	1.4910	1.5215	0.0245	2.2078	7.1371	0.2151	0.2452	0.5119	0.0869	0.8725	0.7099
		<i>P</i>	0.5163	0.3465	0.3427	0.8900	0.2756	0.1162	0.6884	0.6696	0.5486	0.7960	0.4489	0.4882
I × F	1	F	3.4881	0.0783	0.1189	3.4694	0.2161	0.1989	0.0249	0.4181	0.2573	0.2303	0.8639	0.0033
		<i>P</i>	0.2024	0.8058	0.7631	0.2036	0.6877	0.6993	0.8892	0.5842	0.7953	0.6787	0.4508	0.9591
I × Site [F]	2	F	1.0735	5.2129	5.5526	0.4314	11.1452	7.7346	3.3002	5.0217	3.7789	2.0440	9.1993	9.3571
		<i>P</i>	0.3538	0.0132	0.0104	0.6545	0.0004	0.0026	0.0542	0.0151	0.0374	0.1515	0.0011	0.0010

Appendix Table 3.7: Results of a 2-way ANOVA for floodplain type (AL and BW), incubation period (control, 40 h, 8 d), and interaction term for the distribution of total Al and Fe in sequentially extracted fractions (Paludan and Jensen 1995) (**Table 3.9**). Data were log transformed where necessary to meet the assumptions of normality. Significant effects and interaction terms ($P < 0.05$) are shown in bold.

Al and Fe (g m ⁻²)			Distribution of Al and Fe in soil fractions											
	d. f.		H ₂ O		BD		NaOH		HA		HCl		Res	
			Al	Fe	Al	Fe	Al	Fe	Al	Fe	Al	Fe	Al	Fe
Floodplain type, F	1	F	0.0014	0.4475	0.3578	20.3181	0.1214	0.5793	15.4385	26.9253	1.5054	2.5331	2.2680	2.8421
		P	0.9740	0.5724	0.6105	0.0459	0.7607	0.5261	0.0591	0.0352	0.3447	0.2525	0.2449	0.2339
Site [F]	2	F	11.1668	10.3977	11.2383	6.2002	4.5098	9.3701	0.1269	6.8422	14.8862	15.9911	15.2433	21.0406
		P	0.0188	0.0101	0.0163	0.0461	0.0859	0.0242	0.8842	0.0217	0.0092	0.0101	0.0105	0.0052
Incubation, I	1	F	21.7885	11.1876	17.1963	0.3250	15.4412	2.0259	0.4889	1.2557	1.0636	10.1877	1.2125	0.1909
		P	0.0071	0.0230	0.0109	0.7400	0.0131	0.2468	0.6457	0.3774	0.4262	0.0269	0.3876	0.8333
I × F	1	F	1.6274	1.5289	1.8262	0.6950	0.3578	0.2353	0.0821	2.6863	1.1365	4.7313	1.0854	4.9921
		P	0.3040	0.3212	0.2732	0.5508	0.7195	0.8005	0.9227	0.1821	0.4066	0.0883	0.4202	0.0818
I × Site [F]	2	F	1.3221	0.2549	0.8087	0.6546	1.3431	0.9719	4.5723	0.1899	0.7852	1.7591	1.3789	1.0817
		P	0.2861	0.9042	0.5302	0.6286	0.2787	0.4385	0.0057	0.9417	0.5445	0.1652	0.26666	0.3844

CHAPTER 4:

Appendix Figure 4.1: Maximum-parsimony tree showing phylogenetic relationships of bacterial 16S rDNA sequences cloned from soils from one alluvial (AL) (Wateree R., SC) and one blackwater (BL) (Black R., NC) FF, to related sequences from GenBank: **(a)** *Acidobacteria*; **(b)** *Alpha-* and *Gamma-proteobacteria*; **(c)** *Actinobacteria*, *Bacteroidetes*, *Chlorofelxi*, *Fimicutes*, *Gemmatimonadetes*, *Beta-*, and *Delta-proteobacteria*.

Appendix Figure 4.1 a

Appendix Figure 4.1 b

Appendix Figure 4.1 c

Appendix Figure 4.2: Maximum-parsimony tree showing phylogenetic relationships of fungal ITS sequences cloned from soils from one alluvial (AL) (Wateree R., SC) and one blackwater (BL) (Black R., NC) FF, to related sequences from GenBank: **(a)** *Ascomycota*; **(b)** *Basidiomycota*, *Chytridiomycota*, and *Zygomycota*.

Appendix Figure 4.2 a

Appendix Figure 4.2 b

Appendix Figure 4.3. Maximum-parsimony tree showing phylogenetic relationships of bacterial 16S rDNA sequences cloned from soils from ridge and swale microsite elevations in four alluvial (AL) (Wateree R., SC; Pee Dee R., SC; Nottaway R., VA; Ocmulgee R., GA) and four blackwater (BL) (Black R., NC; Drowning Cr., NC; Satilla R., GA; Salkehatchie R., SC) FFs, to related sequences from GenBank: **(a)** *Actinobacteria* *Bacteroidetes*, *Fimicutes*; **(b)** *Acidobacteria*; **(c)** *Chlorofelxi*, *Nitrospirae*; *Alpha-*, *Beta-*, and *Gamma-proteobacteria*.

Appendix Figure 4.3 a

Appendix Figure 4.3 b

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Appendix Figure 4.4. Maximum-parsimony tree showing phylogenetic relationships of fungal ITS sequences cloned from soils from ridge and swale microsites in four alluvial (AL) (Wateree R., SC; Pee Dee R., SC; Nottaway R., VA; Ocmulgee R., GA) and four blackwater (BL) (Black R., NC; Drowning Cr., NC; Satilla R., GA; Salkehatchie R., SC) FFs, to related sequences from GenBank: **(a)** *Ascomycota*; **(b)** *Basidiomycota*, *Chytridiomycota*, and *Zygomycota*.

Appendix Figure 4.4 a

Appendix Figure 4.4 b

Appendix Table 4.1. Identities of (a) bacterial 16S rDNA, and (b) fungal ITS rDNA, OTUs in clone libraries in soils collected from one alluvial (AL) (Wateree R., SC) and one blackwater (BW) (Black R., NC) FF.

Appendix Table 4.1a

out	Access. #	Score	% Sim.	Phylum	Class	Order	Family	Genus	BL	WT	Total
1-TB-463	GQ369128	931/98		Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	11	21	32
2-TB-188	HM748653	1461/95		Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae	Solibacter	13	7	20
3-TB-27	HM748709	885/96		Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		12	4	16
4-TB-212	HM748695	1731/95		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		11	5	16
5-TB-253	AB531426	1764/95		Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	3	10	13
6-TB-237	AY234592	1688/95		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		7	3	10
7-TB-245	AF409023	1629/93		Proteobacteria	Gammaproteobacteria	Chromatiales			2	6	8
8-TB-71	AF498722	806/94		Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylocystis	7		7
9-TB-100	EF074997	870/95		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3	4	7
10-TB-295	AF498727	1458/91		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		6	1	7
11-TB-209	JF510471	1459/90		Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	Aciditerrimonas	7		7
12-TB-349	FJ459988	813/97		Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		4	3	7
13-TB-243	AY234636	1757/95		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		6		6
14-TB-273	AY234650	1275/92		Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	5	1	6
15-TB-453	HM748732	942/97		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			5	5
16-TB-33	NR-025582	713/91		Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		2	2	4
17-TB-40	AM503077	985/99		Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	4		4
18-TB-371	FJ529710	784/94		Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Actinomadura		4	4
19-TB-376	HQ597220	937/99		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			4	4
20-TB-439	GU187030	652/90		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			4	4
21-TB-404	AB268315	575/86		Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geothermobacter		4	4
22-TB-292	FJ605267	1264/92		Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	3		3

23-TB-310	AY234578	473/84	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			3	3
24-TB-280	HM748732	1245/88	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	1	3
25-TB-486	AF421890	514/84	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Sorangium		3	3
26-TB-289	AF498753	1519/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			3	3
27-TB-230	EF019370	1546/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		2	1	3
28-TB-488	AY337603	1003/98	Proteobacteria	Betaproteobacteria	Burkholderiales			0	3	3
29-TB-204	HM748714	1596/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1	2	3
30-TB-462	GU187027	756/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
31-TB-46	FN433469	850/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocapsa	2		2
32-TB-264	FJ469913	1546/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
33-TB-304	AM412118	745/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Chelatococcus		2	2
34-TB-382	HM748732	795/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
35-TB-387	AY673181	765/95	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae			2	2
36-TB-414	JQ357003	837/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
37-TB-487	AF498714	822/93	Proteobacteria	Alphaproteobacteria	Rhizobiales				2	2
38-TB-14	AY673303	811/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	1	2
39-TB-444	AB660496	817/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
40-TB-461	AB470422	760/94	Proteobacteria	Alphaproteobacteria	Rhizobiales				2	2
41-TB-41	AF150790	752/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylocystis	2		2
42-TB-157	EF219138	636/87	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
43-TB-270	AY234728	1310/90	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		2		2
44-TB-328	AF493635	894/98	Bacteroidetes	Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Flavobacterium		2	2
45-TB-363	GU187033	665/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
46-TB-360	AF328857	758/93	Proteobacteria	Deltaproteobacteria	Desulfobacterales				2	2
47-TB-426	HM154525	429/83	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales				2	2
48-TB-186	AF498727	1424/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
49-TB-265	AY234650	1356/89	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		2		2
50-TB-189	AY548785	1646/94	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	2		2
51-TB-314	EU438962	641/91	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella		2	2
52-TB-416	JQ357272	937/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
53-TB-258	HM748653	1507/92	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae	Solibacter	2		2
54-TB-218	HM748695	1520/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2

55-TB-405	FJ711211	994/99	Proteobacteria	Alphaproteobacteria	Rhodospirillales				2	2
56-TB-5	AB568111	599/90	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	1		1
57-TB-6	AJ416913	815/94	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	1		1
58-TB-12	NR-025582	632/88	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Stella	1		1
59-TB-13	AF498741	828/95	Proteobacteria	Alphaproteobacteria	Rhizobiales			1		1
60-TB-15	GU237174	564/89	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Thermosporotrichaceae	Thermosporothrix	1		1
61-TB-17	AY234641	472/84	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales			1		1
62-TB-21	AB568111	686/91	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	1		1
63-TB-25	DQ517128	728/91	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
64-TB-35	AF498744	785/93	Proteobacteria	Alphaproteobacteria	Rhizobiales			1		1
65-TB-48	JF274927	972/99	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Delftia	1		1
66-TB-51	AJ536864	1000/99	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae	Holophaga	1		1
67-TB-53	AF498714	741/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
68-TB-54	AB618788	634/89	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	1		1
69-TB-55	AY234650	800/93	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
70-TB-59	AY234434	859/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
71-TB-67	FJ528273	793/93	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
72-TB-70	FJ870384	760/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
73-TB-73	FR666706	854/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Bryocella	1		1
74-TB-74	JQ358447	795/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
75-TB-84	EF516661	621/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
76-TB-90	JF508369	721/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Microvirga	1		1
77-TB-93	EU849259	1009/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
78-TB-98	JF733443	359/81	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodomicrobium	1		1
79-TB-101	EU628785	989/99	Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae		1		1
80-TB-102	AY234475	664/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
81-TB-111	HM748653	739/91	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1
82-TB-118	EU849300	671/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
83-TB-119	EU849328	684/88	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Actinomadura	1		1
84-TB-121	EF075860	665/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
85-TB-124	EU044271	752/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
86-TB-125	EU044209	725/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1

87-TB-126	HQ598475	863/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
88-TB-127	EU044525	776/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylocystis	1		1
89-TB-128	FN600563	680/89	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
90-TB-144	FJ228278	992/98	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae		1		1
91-TB-164	EU044403	544/85	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Stella	1		1
92-TB-167	AB121773	518/85	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
93-TB-169	EF075418	1022/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
94-TB-171	FR732377	869/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
95-TB-175	AM773622	961/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
96-TB-177	HM062465	693/93	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1
97-TB-182	AF498717	1458/91	Proteobacteria	Alphaproteobacteria	Rhizobiales			1		1
98-TB-184	EU299834	1037/92	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		1		1
99-TB-185	HQ598817	1648/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
100-TB-187	HQ598768	778/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
101-TB-190	HQ598303	728/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
102-TB-191	FJ166826	977/95	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1
103-TB-192	NR-043557	1243/94	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	1		1
104-TB-194	HQ599038	1304/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
105-TB-195	AF376024	1690/95	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Acidisphaera	1		1
106-TB-202	AY387306	1469/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae		1		1
107-TB-207	AF498719	1605/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
108-TB-210	HQ538674	1855/98	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	1		1
109-TB-211	HQ598850	1500/93	Acidobacteria	Acidobacteria				1		1
110-TB-214	AF293379	1463/91	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
111-TB-216	AY234650	1363/89	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
112-TB-219	EU044569	1295/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	1		1
113-TB-222	AY234650	1424/90	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		1		1
114-TB-224	FM203302	1738/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium	1		1
115-TB-225	AY234650	1216/87	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae		1		1
116-TB-227	FJ605267	1205/90	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	1		1
117-TB-228	AY234473	1391/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
118-TB-241	FJ870384	1408/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1

119-TB-247	HQ995659	992/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
120-TB-254	FJ889227	780/82	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
121-TB-256	FJ870380	1866/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
122-TB-261	EF663602	959/87	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	1		1
123-TB-263	HQ598858	1382/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
124-TB-272	FN178370	1050/96	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dyella	1		1
125-TB-275	AY234512	1626/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
126-TB-277	AY234592	935/86	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
127-TB-278	HM748676	1478/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
128-TB-281	AY234636	1818/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
129-TB-283	AY234588	1537/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
130-TB-291	GU187038	959/84	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
131-TB-293	EF073309	1266/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	1		1
132-TB-296	HM748653	1255/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
133-TB-299	AY234650	856/98	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1	1
134-TB-303	NR-024727	809/96	Actinobacteria	Actinobacteridae	Actinomycetales	Sporichthyaceae	Sporichthya		1	1
135-TB-307	FJ228362	837/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
136-TB-311	HM748708	821/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
137-TB-313	AB659723	828/96	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas		1	1
138-TB-317	EF020331	774/95	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae			1	1
139-TB-318	JF319172	843/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
140-TB-322	EF074903	876/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
141-TB-327	EF018500	833/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae			1	1
142-TB-330	HQ162740	664/91	Chloroflexi	Chloroflexi	Thermogemmatisporales	Thermogemmatisporaceae			1	1
143-TB-331	AY436815	870/99	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium		1	1
144-TB-332	HQ018238	603/99	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae			1	1
145-TB-333	FJ484524	865/99	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Syntrophus		1	1
146-TB-334	HM062348	760/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
147-TB-337	HQ684395	773/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae			1	1
148-TB-341	AY043639	571/90	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1	1
149-TB-351	FR687065	765/93	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae			1	1
150-TB-357	FR732382	846/97	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae			1	1

151-TB-365	FJ694258	857/98	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter		1	1
152-TB-367	HQ598715	865/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
153-TB-370	JN869094	881/99	Proteobacteria	Gammaproteobacteria	Chromatiales				1	1
154-TB-372	GU300424	789/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae			1	1
155-TB-374	JQ357287	835/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
156-TB-377	HM062084	848/98	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae			1	1
157-TB-385	NR-042408	811/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Rhodoblastus		1	1
158-TB-389	AB240301	887/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
159-TB-390	EF613852	819/97	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae			1	1
160-TB-395	HM469595	765/96	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophorhabdaceae			1	1
161-TB-406	FJ166571	549/100	Acidobacteria	Acidobacteria					1	1
162-TB-407	AB657993	841/98	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae			1	1
163-TB-408	EU445220	859/99	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae			1	1
164-TB-409	JF119488	658/92	Proteobacteria	Betaproteobacteria	Xanthomonadales	Xanthomonadaceae			1	1
165-TB-411	HM270195	808/98	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	Acidimicrobium		1	1
166-TB-417	HQ598197	1011/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
167-TB-422	GU374396	869/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
168-TB-423	AB293377	815/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
169-TB-429	HM445240	1035/99	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Thiohalospira		1	1
170-TB-433	HM228608	833/99	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae			1	1
171-TB-434	EF020026	741/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
172-TB-436	AM491119	830/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
173-TB-440	EU132462	658/89	Proteobacteria	Deltaproteobacteria					1	1
174-TB-441	FJ037090	774/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
175-TB-442	JF833865	881/99	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas		1	1
176-TB-443	NR-043075	817/99	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter		1	1
177-TB-445	AY795691	739/96	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae			1	1
178-TB-448	FJ166859	695/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
179-TB-452	EF075870	1050/99	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae			1	1
180-TB-470	EU160084	883/99	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae			1	1
181-TB-476	HQ236051	1011/99	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dyella		1	1
182-TB-477	AB658028	1002/98	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae			1	1

183-TB-481	HQ674934	863/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
184-TB-485	JN389687	922/99	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1	1
185-TB-31	GQ402743	634/95	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae		1		1
186-TB-269	JF904128	723/99	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
187-TB-373	EF665504	948/99	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae	Holophaga		1	1

Appendix Table 4.1b

OTU	Access. #	Score % Sim.	Phylum	Subphylum	Class	Order	Family	Genus	BL	WT	Total
1-TF-67	FN428924	926/100	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales		Cryptococcus	18	8	26
2-TF-213	JF439485	1138/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	3	15	18
3-TF-22	EU598156	1037/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	10	2	12
4-TF-127	DQ777988	1051/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	9	1	10
5-TF-216	AY312985	1088,98	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Hebeloma		9	9
6-TF-286	EU784211	909/95	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Entolomataceae	Entoloma		8	8
7-TF-37	EU598194	752/91	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	6	2	8
8-TF-381	AJ557830	1059/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	6	2	8
9-TF-211	EU819478	1116/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria	3	5	8
10-TF-61	JN943015	1062/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	7		7
11-TF-222	AB176571	1081/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales		Daedaleopsis		6	6
12-TF-232	AY656948	870/92	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae			6	6
13-TF-123	HQ157874	750/92	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae		5		5
14-TF-254	AJ633598	1050/97	Basidiomycota							5	5
15-TF-141	JF834358	896/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	5		5
16-TF-109	EU598162	983/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	5		5
17-TF-175	FJ554007	965/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	0	4	4
18-TF-225	JF908544	961/95	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Pezizaceae	Peziza		4	4
19-TF-198	FJ554185	826/93	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae		3	1	4
20-TF-263	GU934592	1053/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales	Corticaceae	Phanerochaete		3	3
21-TF-134	EU819480	957/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	3		3
22-TF-3	U57496	715/91	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae	Arachnopeziza	3		3
23-TF-159	HQ714730	939/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	3		3
24-TF-206	HM488478	902 98	Ascomycota	Pezizomycotina	Leotiomycetes				1	2	3
25-TF-75	HQ630960	1051/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	3		3
26-TF-315	HM176560	1079/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	2	1	3

27-TF-318	EU819493	1140/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula		3	3
28-TF-346	FJ710188	955/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Boletales	Astraeaceae	Astraeus		3	3
29-TF-311	EU167594	966/99	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella		3	3
30-TF-320	GU479272	928/94	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Hymenogastraceae	Hymenogaster		3	3
31-TF-199	HM069444	893/98	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales			1	1	2
32-TF-96	JF735304	987/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Ilyonectria	2		2
33-TF-45	EU598193	821/92	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	2		2
34-TF-60	EU819485	917/95	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	2		2
35-TF-81	FJ553813	992/99	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Dermateaceae		2		2
36-TF-170	AY606960	640/88	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	2		2
37-TF-85	AB218198	808/92	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales			2		2
38-TF-100	EF694630	968/99	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	2		2
39-TF-155	FJ441036	754/90	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Amanitaceae	Amanita	2		2
40-TF-234	AB638487	1081/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella		2	2
41-TF-244	JN159575	874/92	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Psathyrellaceae	Coprinellus		2	2
42-TF-364	FJ904180	1107/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Naucoria		2	2
43-TF-5	EF419996	1002/99	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Cucurbitariaceae		1		1
44-TF-6	HQ608068	924/96	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Chaunopycnis	1		1
45-TF-18	HQ021913	1018/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Cordycipitaceae		1		1
46-TF-19	EU598153	992/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
47-TF-20	AB111493	990/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Mariannaea	1		1
48-TF-24	GU827485	887/98	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Capnodiales	Ramichloridium	1		1
49-TF-25	GU566205	985/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Dothideomycetidae	Fusarium	1		1
50-TF-31	HQ647324	1050/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	1		1
51-TF-36	EU273512	976/98	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Entolomataceae	Clitopilus	1		1
52-TF-38	GQ379737	1005/98	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Tuberaceae	Tuber	1		1
53-TF-40	EU523589	462/81	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	1		1
54-TF-42	HQ222870	488/84	Ascomycota	Pezizomycotina	Geoglossomycetes	Geoglossales	Geoglossaceae	Geoglossum	1		1
55-TF-47	JF273531	981/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	1		1
56-TF-51	HQ211517	817/94	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae	Rhizoscyphus	1		1
57-TF-56	JF273512	771/94	Basidiomycota	Agaricomycotina	Agaricomycetes	Boletales	Boletaceae		1		1
58-TF-57	FJ903291	1011/99	Ascomycota	Pezizomycotina	Sordariomycetes	Coniochaetales	Coniochaetaceae	Lecythophora	1		1

59-TF-58	HQ157959	1040/99	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	1		1
60-TF-62	HM142723	920/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Amanitaceae	Amanita	1		1
61-TF-71	GQ359820	586/88	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae		1		1
62-TF-72	EU819480	880/94	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	1		1
63-TF-73	GU566270	950/97	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Massariaceae		1		1
64-TF-74	FJ553334	569/86	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Agaricomycetidae		1		1
65-TF-79	GQ272612	872/97	Ascomycota	Pezizomycotina	Leotiomycetes				1		1
66-TF-88	GU990359	531/84	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria	1		1
67-TF-89	FJ553154	941/95	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae		1		1
68-TF-91	HQ630279	1075/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae		1		1
69-TF-94	HM239883	795/92	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales			1		1
70-TF-95	EF619640	1042/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Cantharellales	Botryobasidiaceae	Botryobasidium	1		1
71-TF-101	HQ608068	946/97	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Chaunopycnis	1		1
72-TF-102	FN428906	725/98	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales			1		1
73-TF-103	HQ631046	850/99	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales			1		1
74-TF-104	HQ631007	963/97	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	1		1
75-TF-112	GU046494	612/92	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	1		1
76-TF-114	JN021025	990/98	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Ganodermataceae	Ganoderma	1		1
77-TF-115	JF439515	730/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	1		1
78-TF-121	HQ608141	1013/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Chaunopycnis	1		1
79-TF-131	FJ553896	713/92	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales			1		1
80-TF-132	GQ241283	1035/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Paecilomyces	1		1
81-TF-137	AY456348	911/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	1		1
82-TF-146	EU819437	830/93	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
83-TF-151	HQ157874	595/87	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae		1		1
84-TF-154	AY456373	1038/98	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	Clavaria	1		1
85-TF-163	AJ889946	721/90	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	1		1
86-TF-168	EF040864	939/97	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales			1		1
87-TF-169	GU174357	979/99	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae		1		1
88-TF-174	FJ596782	1038/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Gymnopus	1		1
89-TF-176	L14526	811/93	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Talaromyces	1		1
90-TF-179	EU240130	889/95	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae		1		1

91-TF-180	AB208110	1013/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Tolypocladium	1		1
92-TF-182	EU819480	765/98	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	1		1
93-TF-184	FJ538960	739/91	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales			1		1
94-TF-188	EU819511	917/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
95-TF-189	EF218811	547/86	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
96-TF-190	FJ553813	721/90	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales			1		1
97-TF-197	AY312985	1051/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Hebeloma		1	1
98-TF-203	DQ469743	1027/98	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales				1	1
99-TF-212	JN942178	632/99	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Lasiosphaeris		1	1
100-TF-233	HM488599	712/96	Ascomycota	Pezizomycotina	Dothideomycetes	Venturiales	Venturiaceae			1	1
101-TF-236	AB470888	1114/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Strophariaceae	Pholiota		1	1
102-TF-239	FJ553238	990/95	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Sporidiobolales				1	1
103-TF-241	JQ268558	719/94	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae	Hymenoscyphus		1	1
104-TF-242	GQ996133	761/98	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales				1	1
105-TF-243	DQ974762	1068/98	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula		1	1
106-TF-245	EU520251	1044/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Psathyrellaceae	Psathyrella		1	1
107-TF-247	AB542112	1027/97	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae			1	1
108-TF-248	AJ585505	939/94	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Strophariaceae	Galerina		1	1
109-TF-250	AF444482	887/99	Basidiomycota	Agaricomycotina	Tremellomycetes	Cystofilobasidiales				1	1
110-TF-255	GU566228	824/93	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales				1	1
111-TF-256	AJ244263	845/92	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Trimmatostroma		1	1
112-TF-257	GU931742	935/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Cylindrium		1	1
113-TF-258	EU819461	1066/98	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Pezizaceae	Peziza		1	1
114-TF-262	AF444482	569/92	Basidiomycota	Agaricomycotina	Tremellomycetes	Cystofilobasidiales				1	1
115-TF-273	HQ630337	739/97	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella		1	1
116-TF-279	EU819478	760/89	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria		1	1
117-TF-284	FM999536	643/86	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales				1	1
118-TF-299	AF255099	1074/100	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Ganodermataceae	Ganoderma		1	1
119-TF-302	AB542112	965/94	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae			1	1
120-TF-304	AJ301970	891/97	Ascomycota	Pezizomycotina	Sordariomycetes	Glomerellales	Glomerellaceae	Glomerella		1	1
121-TF-305	EU552110	981/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Bionectriaceae	Bionectria		1	1
122-TF-306	HQ713763	957/98	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Didymosphaeria		1	1

123-TF-310	FJ612884	684/91	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales				1	1
124-TF-312	AJ875391	933/97	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales				1	1
125-TF-314	GQ411315	1022/98	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae	Dimorphospora		1	1
126-TF-319	DQ421269	1011/96	Basidiomycota	Agaricomycotina	Agaricomycetes					1	1
127-TF-323	AF444387	819/97	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales				1	1
128-TF-324	GU062271	789/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales				1	1
129-TF-326	HQ608097	889/96	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae			1	1
130-TF-327	AF346545	1005/98	Ascomycota	Pezizomycotina	Sordariomycetes	Xylariales	Amphisphaeriaceae	Amphisphaeria		1	1
131-TF-328	HQ829334	789/93	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales				1	1
132-TF-331	HQ829334	778/93	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales				1	1
133-TF-343	HQ623459	913/98	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales				1	1
134-TF-347	AJ879645	628/88	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Helvellaceae			1	1
135-TF-349	HQ162263	928/99	Ascomycota	Pezizomycotina	Dothideomycetes;	Capnodiales	Mycosphaerellaceae	Mycosphaerella		1	1
136-TF-355	HQ608027	953/99	Ascomycota	Pezizomycotina	Sordariomycetes	Microascales	Halosphaeriaceae	Periconia		1	1
137-TF-357	GQ280647	979/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Calonectria		1	1
138-TF-359	AY312985	920/93	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Hebeloma		1	1
139-TF-360	EU266591	1068/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Pochonia		1	1
140-TF-362	DQ485660	1009/99	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	Rhizophydium		1	1
141-TF-367	JQ065032	989/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Pluteaceae	Pluteus		1	1
142-TF-369	EU819478	782/91	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria		1	1
143-TF-370	DQ273336	924/98	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales				1	1
144-TF-371	GQ166879	1033/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Boletales	Boletaceae	Boletus		1	1
145-TF-372	EU784411	1094/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Boletales	Sclerodermataceae	Scleroderma		1	1
146-TF-380	HM595561	1103/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Psathyrellaceae	Coprinellus		1	1
147-TF-382	DQ093730	774/94	Zygomycota	Mucoromycotina		Mortierellales				1	1

Appendix Table 4.2. Identities of (a) bacterial 16S rDNA, and (b) fungal ITS rDNA, OTUs in clone libraries in soils collected from four alluvial (WT: Wateree R., SC; PD: Pee Dee R., SC; NT: Nottaway R., VA; and OC: Ocmulgee R., GA) and four blackwater (BL: Black R., NC; DC: Drowning Cr., NC; ST: Satilla R., GA; SK: Salkehatchie R., SC) FFs.

Appendix Table 4.2a

OTU	Accession #	Score	%Sim.	Phylum	Class	Order	Family	Genus	Ridge	Swale	Total
1-MB-559	AB531426	1997/98		Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	6	11	17
2-MB-367	HM748695	1783/97		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		4	8	12
3-MB-383	HM748732	1714/93		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3	5	8
4-MB-385	AF409023	1807/95		Proteobacteria	Gammaproteobacteria	Chromatiales			2	6	8
5-MB-526	GQ369128	1760/95		Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	3	4	7
6-MB-513	HM748653	1993/96		Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae	Solibacter	4	2	6
7-MB-164	AY234650	1506/91		Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		4	2	6
8-MB-32	EU938323	1893/95		Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	3	2	5
9-MB-443	AF498753	1923/96		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3	2	5
10-MB-440	AF498727	1672/93		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	3	5
11-MB-627	NR-043898	1764/95		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Edaphobacter	3	1	4
12-MB-350	AY234636	1978/97		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	2	4
13-MB-506	HM748732	1770/95		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2	1	3
14-MB-29	HM748695	1602/91		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3		3
15-MB-641	D16209	1387/91		Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Rhodocyclus	3		3
16-MB-453	AB250621	1657/92		Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodomicrobium		3	3
17-MB-392	AF498727	1472/91		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	2	3
18-MB-210	AY234475	1456/93		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3		3
19-MB-441	GU187030	1526/91		Acidobacteria	Acidobacteria	Holophagales	Holophagaceae		1	2	3
20-MB-68	HM748754	1709/98		Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		3		3

21-MB-196	HM748695	1814/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
22-MB-86	HM748653	1681/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		2		2
23-MB-386	GU129927	1842/96	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	1	1	2
24-MB-112	AB111107	1626/94	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		2		2
25-MB-231	FJ190062	1068/89	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	Anaeromyxobacter	2		2
26-MB-447	AB552842	1472/89	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Sulfuritalea		2	2
27-MB-35	HM748714	1674/93	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		2		2
28-MB-668	EU331408	1319/88	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	Anaeromyxobacter	2		2
29-MB-90	HM748715	1687/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
30-MB-59	AF482687	1587/92	Proteobacteria	Deltaproteobacteria	Myxococcales			2		2
31-MB-114	HM748754	1496/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
32-MB-455	HM748709	1851/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		1	1	2
33-MB-630	GU187033	1428/89	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae		2		2
34-MB-273	AB598278	941/83	Chloroflexi	Chloroflexi	Anaerolineales	Anaerolineaceae		2		2
35-MB-444	AY234551	1685/93	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1	1	2
36-MB-381	AY234546	1620/96	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae			2	2
37-MB-639	AY234650	1923/97	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		2		2
38-MB-522	AB681929	1842/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1	1	2
39-MB-359	HM748732	1698/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	1	2
40-MB-255	AB669481	1223/87	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
41-MB-361	HM748709	1701/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae			2	2
42-MB-469	AY234546	1701/94	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae			2	2
43-MB-496	AM503077	1615/98	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1	1	2
44-MB-549	FP929063	1061/89	Nitrospirae	Nitrospirae	Nitrospirales	Nitrospiraceae			2	2
45-MB-565	DQ490442	1615/95	Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae			2	2
46-MB-23	HM748695	1615/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	1	2
47-MB-57	FJ190062	1173,86	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	Anaeromyxobacter	2		2
48-MB-51	HM748739	1360/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
49-MB-148	AB681301	1799/95	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia		2	2
50-MB-456	HM748732	1666/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	1	2
51-MB-398	HQ995662	1895/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
52-MB-343	HM748739	1652/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1	1	2

53-MB-389	GU187030	1229/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
54-MB-263	AB268315	1134/86	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geothermobacter		2	2
55-MB-162	HM748653	1607/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		2		2
56-MB-339	FJ870384	1637/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			2	2
57-MB-357	EU938323	1731/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		1	1	2
58-MB-1	DQ35518	1652/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
59-MB-2	AF200698	1729/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Acidobacterium	1		1
60-MB-3	GU205283	1526/91	Proteobacteria	Deltaproteobacteria				1		1
61-MB-5	AF498722	1430/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae		1		1
62-MB-6	JF916701	1816/95	Proteobacteria	Betaproteobacteria	Burkholderiales			1		1
63-MB-7	EU930870	1443/90	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Parvibaculum	1		1
64-MB-8	AB120965	1605/92	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Aquamonas	1		1
65-MB-11	EF072379	1327/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
66-MB-12	EF075894	1038/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
67-MB-14	NR-042107	1650/93	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	Methylobacter	1		1
68-MB-15	GU785010	1797/95	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dyella	1		1
69-MB-16	AY234726	1587/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
70-MB-17	HM061991	1053/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
71-MB-20	NR-042341	1735/94	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae		1		1
72-MB-24	EF019370	1609/93	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
73-MB-27	HQ995662	1387/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
74-MB-28	GU205304	1423/92	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae		1		1
75-MB-30	AY234512	1683/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
76-MB-31	EU359919	1120/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
77-MB-33	JN409187	1531/96	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae		1		1
78-MB-34	HM748695	1707/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
79-MB-37	FJ166294	1033/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
80-MB-40	EF612346	1666/94	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae		1		1
81-MB-41	DQ450715	1310/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
82-MB-43	AM935097	1881/95	Proteobacteria	Deltaproteobacteria	Syntrophobacteriales			1		1
83-MB-44	AF498710	1895/97	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		1		1
84-MB-45	GU207881	1585/91	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Polyangium	1		1

85-MB-47	AF077729	1749/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	1		1
86-MB-55	EU043719	1685/93	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
87-MB-56	EF019707	1607/95	Proteobacteria	Alphaproteobacteria	Rhizobiales			1		1
88-MB-58	HM061886	1967/97	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae		1		1
89-MB-62	AM162403	1256/88	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
90-MB-63	AY234497	1626/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		1		1
91-MB-64	EF020321	1426/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae		1		1
92-MB-67	AY395352	1685/93	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
93-MB-69	HM748695	1613/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
94-MB-75	EF019164	1428/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
95-MB-78	AY234650	1461/90	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
96-MB-79	AJ582047	1768/95	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae		1		1
97-MB-81	AY234588	1803/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
98-MB-83	AJ277699	1458/94	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobineae		1		1
99-MB-87	FJ528270	1831/96	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1		1
100-MB-89	EU202789	1877/95	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae		1		1
101-MB-97	AY234434	1703/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
102-MB-98	FJ517007	1646/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
103-MB-102	JF510471	1391/90	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobineae		1		1
104-MB-103	EU445211	1323/90	Proteobacteria	Deltaproteobacteria				1		1
105-MB-105	AF376024	1770/96	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Acidisphaera	1		1
106-MB-113	EF019150	1688/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
107-MB-115	AY234538	1653/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
108-MB-117	EF019164	1308/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
109-MB-120	EF203409	1343/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
110-MB-121	GQ402716	1286/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae		1		1
111-MB-126	AB426201	1515/93	Proteobacteria	Betaproteobacteria	Burkholderiales			1		1
112-MB-127	EF516921	1535/90	Chloroflexi	Chloroflexi	Ktedonobacterales	Ktedonobacteraceae		1		1
113-MB-135	HM062036	1485/90	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1
114-MB-137	EF019194	1452/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
115-MB-146	GQ369103	1604/93	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Agromonas	1		1
116-MB-149	EU299862	1040/91	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1

117-MB-150	AY395368	1186/93	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
118-MB-152	AF482687	1254/88	Proteobacteria	Deltaproteobacteria	Myxococcales			1		1
119-MB-155	EF111055	1094/88	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		1		1
120-MB-159	AB252927	1365/91	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae		1		1
121-MB-160	AY234531	1317/90	Proteobacteria	Gammaproteobacteria				1		1
122-MB-165	HE575950	1428/93	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	1		1
123-MB-167	EF018409	1544/94	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae		1		1
124-MB-169	EF074395	1190/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
125-MB-171	FM253597	1572/91	Proteobacteria	Alphaproteobacteria	Rhizobiales			1		1
126-MB-173	AY234640	1376/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
127-MB-179	GU433471	1153/85	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
128-MB-181	DQ419959	1227/87	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1		1
129-MB-184	AY673303	1129/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
130-MB-185	AB201284	1829/96	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1		1
131-MB-187	HM062175	1297/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
132-MB-188	FJ889227	1764/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
133-MB-192	EF019164	1653/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
134-MB-194	AJ519370	1495/91	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae	Holophaga	1		1
135-MB-195	HM062367	1714/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1
136-MB-198	AY607127	1064/88	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	Syntrophobacter	1		1
137-MB-199	AF408985	1788/95	Actinobacteria	Actinobacteria	Actinomycetales			1		1
138-MB-200	EF072647	1074/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
139-MB-205	AM114444	1365/91	Bacteroidetes	Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae		1		1
140-MB-207	EF075552	1491/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae		1		1
141-MB-209	CU919466	1670/97	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
142-MB-211	EF018422	1851/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
143-MB-215	EU445211	1380/92	Proteobacteria	Deltaproteobacteria				1		1
144-MB-216	AJ458492	1140/90	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylosinus	1		1
145-MB-221	GQ203308	1768/96	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiineae		1		1
146-MB-223	EF072661	1249/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
147-MB-226	EF520430	1426/92	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		1		1
148-MB-227	EF019150	1168/87	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1

149-MB-229	EF019009	1570/94	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae		1		1
150-MB-230	HM061991	1352/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
151-MB-236	HM061739	1424/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
152-MB-243	EU440612	1157/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
153-MB-246	HM061759	1417/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
154-MB-249	EU300005	1173/93	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
155-MB-250	AY963511	1042/88	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
156-MB-254	DQ450700	1694/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
157-MB-256	EU359940	1367/93	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae		1		1
158-MB-257	EU680452	1362/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
159-MB-258	DQ450715	1661/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
160-MB-259	EU043719	1639/92	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
161-MB-266	NR-025764	1668/94	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Aquicella	1		1
162-MB-267	EU202832	1236/86	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	1		1
163-MB-268	AJ519407	1439/90	Bacteroidetes	Bacteroidetes		Rhodothermaceae		1		1
164-MB-269	AF49868	1910/96	Proteobacteria	Gammaproteobacteria	Xanthomonadales			1		1
165-MB-271	GU433471	1622/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
166-MB-272	<u>AF498727</u>	1347/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
167-MB-274	HQ730965	1906/97	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1		1
168-MB-276	EF019497	1375/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
169-MB-278	AB531406	1565/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
170-MB-281	HM748732	1524/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
171-MB-282	AY921819	1509/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		1		1
172-MB-284	HM062260	1452/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
173-MB-289	HM062367	1264/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1
174-MB-296	GU433471	1218/90	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
175-MB-297	EF018307	1279/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
176-MB-304	AB191225	1681/98	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1		1
177-MB-311	DQ829647	1430/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
178-MB-312	JF266653	1544/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	1		1
179-MB-315	AF408987	1890/96	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcineae	Microbacteriaceae		1	1
180-MB-316	EF464628	1155/92	Nitrospirae	Nitrospirae	Nitrospirales	Nitrospiraceae			1	1

181-MB-319	FJ535110	1504/91	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae			1	1
182-MB-320	EU193067	1592/91	Proteobacteria	Deltaproteobacteria	Myxococcales	Sorangiiaceae	Polyangiaceae		1	1
183-MB-323	AY921916	1838/95	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1	1
184-MB-328	DQ114945	1731/94	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium		1	1
185-MB-329	AY395395	1345/90	Proteobacteria	Deltaproteobacteria	Myxococcales				1	1
186-MB-331	EU359920	1437/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
187-MB-332	EU665032	1718/98	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae			1	1
188-MB-335	AY963313	1672/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae			1	1
189-MB-337	AY234723	1805/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
190-MB-338	GU187033	1519/91	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae			1	1
191-MB-340	EU445198	1426/91	Proteobacteria	Deltaproteobacteria					1	1
192-MB-341	AJ519365	1242/87	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae	Holophaga		1	1
193-MB-345	AJ518801	1679/93	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae			1	1
194-MB-349	GU167988	1906/97	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae			1	1
195-MB-351	AY673181	1690/93	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae			1	1
196-MB-352	EF019854	1203/86	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae			1	1
197-MB-354	GU172191	1469/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
198-MB-356	HM062040	1495/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
199-MB-358	DQ450764	1604/95	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae			1	1
200-MB-363	FJ542862	1469/90	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio		1	1
201-MB-364	EF018995	1415/90	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1	1
202-MB-365	EU043719	1807/95	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae			1	1
203-MB-369	AJ534615	1437/92	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1	1
204-MB-370	EF018936	1491/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
205-MB-372	AM887757	1493/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella		1	1
206-MB-374	EF020321	1568/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1	1
207-MB-376	AY234503	1816/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1	1
208-MB-379	AJ518771	1583/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1	1
209-MB-382	EF018987	1668/93	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1	1
210-MB-388	EU043719	1628/92	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae			1	1
211-MB-390	EF075624	1325/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
212-MB-391	HM061755	1648/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1

213-MB-393	EU359920	1635/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
214-MB-394	HM061754	1397/92	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae			1	1
215-MB-395	DQ450715	1535/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
216-MB-397	AF293007	1520/94	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia		1	1
217-MB-400	DQ829647	1042/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
218-MB-401	HM062276	1694/96	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae			1	1
219-MB-407	EU440668	1419/92	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae			1	1
220-MB-410	FJ889345	1099/89	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dokdonella		1	1
221-MB-411	AY395390	1208/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
222-MB-413	HM748702	1546/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
223-MB-421	AF281142	1753/95	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium		1	1
224-MB-422	HM748650	1679/93	Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleriaceae			1	1
225-MB-429	HM187222	1371/89	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium		1	1
226-MB-430	AF236003	1821/95	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae			1	1
227-MB-436	EU043855	1408/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes		1	1
228-MB-439	AF498724	1616/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
229-MB-442	X77835	1844/96	Firmicutes	Clostridia	Clostridiales	Clostridiaceae			1	1
230-MB-445	DQ493433	1772/95	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae			1	1
231-MB-446	HM748650	1321/87	Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleriaceae			1	1
232-MB-448	AF408981	1469/96	Proteobacteria	Gammaproteobacteria	Xanthomonadales				1	1
233-MB-449	AJ518781	1755/94	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae			1	1
234-MB-450	DQ829637	1714/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
235-MB-451	AY921812	1949/96	Proteobacteria	Gammaproteobacteria	Xanthomonadales				1	1
236-MB-457	EF018936	1858/95	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
237-MB-459	AM114436	1768/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae			1	1
238-MB-460	EF019150	1635/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae			1	1
239-MB-462	FN811194	1620/91	Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleriaceae			1	1
240-MB-463	AY234420	1801/95	Proteobacteria	Alphaproteobacteria	Rhodospirillales				1	1
241-MB-464	AY395432	1814/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae			1	1
242-MB-465	AJ534617	1452/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae			1	1
243-MB-468	GU983323	1267/93	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae			1	1
244-MB-473	EF019452	1452/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1

245-MB-474	HM062167	1672/94	Acidobacteria	Acidobacteria	Solibacteres	Solibacterales			1	1
246-MB-475	EF074954	1181/93	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1	1
247-MB-476	HM062470	1572/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
248-MB-479	AB672176	1061/91	Nitrospirae	Nitrospirae					1	1
249-MB-480	EF018602	1666/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
250-MB-481	HM062456	1375/93	Acidobacteria	Acidobacteria	Solibacteres	Solibacterales			1	1
251-MB-484	CU922792	1469/92	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1	1
252-MB-487	HM062092	1478/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
253-MB-493	GU120600	1399/96	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae			1	1
254-MB-500	AB303630	1521/93	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia		1	1
255-MB-502	AJ518779	1853/96	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae			1	1
256-MB-504	GU472718	1382/94	Bacteroidetes	Bacteroidia	Cytophagales	Cytophagaceae			1	1
257-MB-505	AJ518803	1502/92	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae			1	1
258-MB-508	EF075146	1214/94	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae			1	1
259-MB-510	EF074811	1120/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
260-MB-512	AY234728	1661/93	Acidobacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae		1	1
261-MB-514	AJ582047	1192/88	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
262-MB-515	X97077	1947/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae			1	1
263-MB-516	EF019194	1345/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
264-MB-519	EF111055	1676/95	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae			1	1
265-MB-521	EU297133	1162/94	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium		1	1
266-MB-527	GQ500694	1797/96	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae			1	1
267-MB-528	GU187025	1496/90	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
268-MB-530	HM062470	1555/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
269-MB-531	HM061759	1476/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
270-MB-532	CU921195	1430/91	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae			1	1
271-MB-533	FJ516999	1620/93	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae			1	1
272-MB-534	HM061754	1727/94	Acidobacteria	Acidobacteria	Solibacteres	Solibacterales			1	1
273-MB-536	JN180107	1705/94	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium		1	1
274-MB-537	HM062457	1720/93	Acidobacteria	Acidobacteria	Solibacteres	Solibacterales			1	1
275-MB-539	AB486908	1186/92	Firmicutes	Clostridia	Clostridiales	Clostridiaceae			1	1
276-MB-545	FJ485076	1650/95	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae			1	1

277-MB-547	EF018851	1297/87	Actinobacteria	Actinobacteria	Actinomycetales	Thermomonosporaceae	Actinocorallia		1	1
278-MB-550	GQ249356	1485/91	Proteobacteria	Deltaproteobacteria	Myxococcales	Sorangiiineae			1	1
279-MB-553	HM061754	1790/95	Acidobacteria	Acidobacteria	Solibacteres	Solibacterales			1	1
280-MB-554	DQ450810	1674/94	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter		1	1
281-MB-556	EU445198	1478/93	Proteobacteria	Deltaproteobacteria	Myxococcales				1	1
282-MB-561	AY234728	1480/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae	Solibacter		1	1
283-MB-567	EU413907	1439/90	Actinobacteria	Actinobacteria	Actinomycetales	Streptomyetaceae	Streptomyces		1	1
284-MB-570	AF524860	1384/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
285-MB-575	HM061856	1352/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
286-MB-576	EF075552	1480/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae			1	1
287-MB-580	HM062175	1589/92	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
288-MB-581	EF018331	1415/89	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae			1	1
289-MB-582	HM062058	1533/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
290-MB-584	AY234726	1666/98	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
291-MB-586	EF019164	717/81	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
292-MB-587	HM062367	1868/96	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae			1	1
293-MB-592	JN590319	1869/96	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia		1	1
294-MB-596	DQ109933	1216/89	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae		1		1
295-MB-599	EF125937	1561/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
296-MB-600	EU680452	985/84	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
297-MB-602	NR-042088	1247/88	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	1		1
298-MB-607	EF020306	1208/88	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae		1		1
299-MB-612	EF019150	1844/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
300-MB-616	HM061936	1755/93	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
301-MB-617	EU440666	1557/94	Proteobacteria	Deltaproteobacteria	Myxococcales			1		1
302-MB-619	EF019026	1118/86	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
303-MB-622	EF019506	1476/91	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
304-MB-626	HM061921	1469/95	Acidobacteria	Acidobacteria	Holophagales	Holophagaceae		1		1
305-MB-634	AY691423	1537/97	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Rhodocyclus	1		1
306-MB-635	HQ023246	1391/93	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	1		1
307-MB-636	AY395390	1755/94	Acidobacteria	Acidobacteria	Solibacterales	Solibacteraceae		1		1
308-MB-637	EF020280	1376/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1

309-MB-638	DQ110051	1338/96	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Pelobacteraceae		1		1
310-MB-642	HM062268	1450/89	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
311-MB-643	AJ582046	1478/91	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
312-MB-645	EU043719	1659/92	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		1		1
313-MB-646	DQ450717	1663/94	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
314-MB-651	EF074384	1496/97	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae		1		1
315-MB-653	GQ369058	1504/95	Actinobacteria	Actinobacteria	Acidimicrobiales	Iamiaceae	Iamia	1		1
316-MB-663	EF665816	1441/97	Proteobacteria	Alphaproteobacteria	Rhizobiales			1		1
317-MB-664	AY395368	1327/96	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		1		1
318-MB-672	EU043922	1131/91	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae		1		1
319-MB-673	AY673350	1075/89	Acidobacteria	Acidobacteria				1		1
320-MB-674	EU665032	1275/92	Acidobacteria	Acidobacteria				1		1

Appendix Table 4.2b

OTU	Accession	Score	% Sim.	Phylum	Subphylum	Class	Order	Family	Genus	RD	SW	Total
1-MF-346	AJ557830	1075/99		Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	36	97	133
2-MF-271	FN428924	935/99		Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales		Cryptococcus	18	12	30
3-MF-266	JF439485	1181/99		Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	5	7	12
4-MF-159	HM176576	1101/99		Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	8		8
5-MF-356	AY854068	569/100		Basidiomycota	Agaricomycotina	Agaricomycetes	Boletales	Boletaceae	Strobilomyces		6	6
6-MF-314	DQ195594	763/86		Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae			6	6
7-MF-49	EU819493	1120/97		Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Amanitaceae	Amanita	6		6
8-MF-26	EU598153	1140/99		Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	2	3	5
9-MF-96	FJ845443	678/85		Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Tricholoma	1	4	5
10-MF-383	HM189876	985/92		Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales				5	5
11-MF-397	HQ873381	425/88		Zygomycota						1	3	4
12-MF-23	AM084698	928/99		Ascomycota	Pezizomycotina	Dothideomycetes			Cenococcum	4	1	5
13-MF-70	EU557361	950/99		Ascomycota	Pezizomycotina	Sordariomycetes	Microascales	Halosphaeriaceae	Sigmoidea	4		4
14-MF-18	EU186277	1266/99		Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Tricholoma	4		4
15-MF-229	EU819478	1267/99		Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria		4	4
16-MF-312	HQ608146	1074/99		Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus		4	4
17-MF-391	GU566216	1016/98		Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium		4	4
18-MF-127	DQ777988	1192/99		Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	3		3
19-MF-21	GU212410	701/93		Ascomycota	Pezizomycotina	Sordariomycetes	Magnaporthales			3		3
20-MF-115	JF439489	1166/99		Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	1	2	3
21-MF-373	JF908140	638/84		Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Inocybaceae	Inocybe		3	3
22-MF-359	HQ630960	1131/99		Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea		3	3
23-MF-74	HQ604626	706/86		Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	3		3
24-MF-315	EU035459	785/92		Ascomycota	Pezizomycotina	Dothideomycetes	Venturiales	Venturiaceae	Venturia		3	3
25-MF-441	JN882326	364/81		Chytridiomycota		Monoblepharidomycetes	Monoblepharidales	Gonapodyaceae	Gonapodya	2		2
							Agaricales					
26-MF-17	HQ604561	593/85		Basidiomycota	Agaricomycotina	Agaricomycetes		Inocybaceae	Inocybe	2		2

27-MF-145	AJ633598	1064/97	Basidiomycota						2		2
28-MF-9	FJ475798	355/82	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales			2		2
29-MF-199	FJ612884	736/94	Ascomycota	Pezizomycotina	Sordariomycetes					2	2
30-MF-277	EU598167	832/91	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula		2	2
31-MF-279	EU598167	1164/100	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula		2	2
32-MF-217	JF735304	989/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Ilyonectria		2	2
33-MF-220	FN397177	492/84	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales				2	2
34-MF-322	FJ000401	948/99	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales		Angulospora		2	2
35-MF-336	GU997932	896/96	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae			2	2
36-MF-420	JF439486	1181/99	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella		2	2
37-MF-11	HQ631003	1013/99	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Davidiellaceae	Cladosporium	1	1	2
38-MF-435	HQ157874	763/92	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae		1	1	2
39-MF-50	EF619863	771/94	Ascomycota	Pezizomycotina	Dothideomycetes	Venturiales			2		2
40-MF-118	HM562160	1136/97	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Pluteaceae	Pluteus	2		2
41-MF-317	HM036644	1072/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	Asterophora		2	2
42-MF-431	DQ431175	846/98	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae	Hymenoscyphus		2	2
43-MF-57	HQ608097	760/98	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	2		2
44-MF-273	FM999581	357/78	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales				2	2
45-MF-293	FJ196771	750/94	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Arnium		2	2
46-MF-5	AF418616	977/93	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
47-MF-12	GU234137	507/84	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	Lyophyllum	1		1
48-MF-16	FJ554007	987/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	1		1
49-MF-19	EU819493	1266/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
50-MF-22	FJ378813	1123/95	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius	1		1
51-MF-28	JF908706	514/83	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
52-MF-36	AY456364	979/94	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
53-MF-37	GU934589	1105/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	1		1
54-MF-40	JN882326	363/81	Chytridiomycota		Monoblepharidomycetes	Monoblepharidales	Gonapodyaceae	Gonapodya	1		1
55-MF-44	EU035406	843/91	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	1		1
56-MF-47	GU566253	1035/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Bionectriaceae	Bionectria	1		1
57-MF-54	FJ403209	580/83	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales		Daedalea	1		1
58-MF-66	FJ589646	994/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Metarhizium	1		1

59-MF-71	HM069466	346/82	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Eurotiomycetidae	Elaphomyces	1		1
60-MF-73	FJ770373	756/90	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreomycetidae	Acremonium	1		1
61-MF-76	EU625924	743/98	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae		1		1
62-MF-79	HQ637272	640/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreomycetidae		1		1
63-MF-80	AJ557830	730/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreomycetidae	Nectria	1		1
64-MF-84	HM176567	1070/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	1		1
65-MF-85	FJ440938	833/93	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales	Sordariomycetidae		1		1
66-MF-95	HM240099	815/91	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	1		1
67-MF-97	AY456364	706/86	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
68-MF-99	FJ876187	1096/96	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Eurotiomycetidae	Elaphomyces	1		1
69-MF-104	EU625924	579/92	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae		1		1
70-MF-105	FJ613023	1074/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreomycetidae	Trichoderma	1		1
71-MF-106	HQ599581	922/96	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales			1		1
72-MF-107	EF029220	918/98	Ascomycota	Pezizomycotina	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	Chloridium	1		1
73-MF-109	AB218096	525/95	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales			1		1
74-MF-111	AM944351	933/98	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Bionectriaceae	Bionectria	1		1
75-MF-112	HQ392603	769/94	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae	Mortierella	1		1
76-MF-113	AJ557830	769/91	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreomycetidae	Nectria	1		1
77-MF-117	AY789432	745/92	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae	Hymenoscyphus	1		1
78-MF-119	EU998926	874/95	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae	Articulospora	1		1
79-MF-140	EU625924	1205/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae		1		1
80-MF-146	AY456348	1208/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulaceae	Russulaceae	Lactarius	1		1
81-MF-150	DQ178166	972/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Ilyonectria	1		1
82-MF-155	JF439497	1016/98	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	1		1
83-MF-156	AY587914	475/82	Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Lasiosphaeria	1		1
84-MF-164	EU784197	800/92	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Elaphomycetaceae	Elaphomyces	1		1
85-MF-167	AJ557830	745/97	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Neonectria	1		1
86-MF-171	GU327518	929/93	Zygomycota	Mucoromycotina		Mortierellales	Mortierellaceae		1		1
87-MF-173	AJ534905	1157/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula	1		1
88-MF-175	AB258369	883/92	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	Paecilomyces	1		1
89-MF-178	FJ554109	588/85	Basidiomycota	Pucciniomycotina	Pucciniomycetes	Pucciniales			1		1
	AJ534912			Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella			
90-MF-181		920/92	Basidiomycota						1		1

91-MF-183	FJ876187	416/90	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Elaphomycetaceae	Elaphomyces	1		1
92-MF-186	JN225930	424/81	Ascomycota	Pezizomycotina	Dothideomycetes				1		1
93-MF-188	JN098488	610/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales			1		1
94-MF-219	HM051159	641/95	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Dothideomycetidae	Capnodiales		1	1
95-MF-228	FM999707	854/91	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales				1	1
96-MF-252	EU449953	606/89	Ascomycota	Pezizomycotina	Dothideomycetes	Venturiales				1	1
97-MF-255	HM189876	1134/96	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Russula		1	1
98-MF-268	EU167594	915/98	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella		1	1
99-MF-291	GU566205	992/99	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreomycetidae	Fusarium		1	1
100-MF-294	HM051159	765/95	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales				1	1
101-MF-313	FJ501561	878/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Ganodermataceae	Ganoderma		1	1
102-MF-316	AY383749	1051/99	Basidiomycota	Pucciniomycotina	Microbotryomycetes		Microbotryomycetidae	Rhodotorula		1	1
103-MF-351	AY312985	1140/99	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Hebeloma		1	1
104-MF-362	EF672298	977/99	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporomycetidae	Pleosporales		1	1
105-MF-372	HM488478	872/96	Ascomycota	Pezizomycotina	Leotiomycetes					1	1
106-MF-378	GU233360	560/84	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae	Astrosporina		1	1
107-MF-381	EU019284	913/98	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Ramularia		1	1
108-MF-388	FJ553813	1007/99	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Dermateaceae			1	1
109-MF-399	EU444535	874/95	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales	Corticaceae	Sistotrema		1	1
110-MF-402	HM189994	1024/94	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella		1	1
111-MF-407	EU520616	438/82	Ascomycota	Pezizomycotina	Dothideomycetes	Venturiales	Venturiaceae			1	1
112-MF-415	AB505408	425/81	Ascomycota	Pezizomycotina	Sordariomycetes	Diaporthales	Valsaceae	Phomopsis		1	1
113-MF-419	HQ634614	632/84	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Chaetothyriomycetidae			1	1
114-MF-424	EF141550	1112/92	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	Lactarius		1	1