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RESEARCH ARTICLE

Soil nitrogen dynamics as an indicator for longleaf pine restoration

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Assessing the status of soil nutrients with their corresponding microbial communities provides important information about degraded soils during the restoration of coastal wet pine forests. Net nitrogen mineralization, nitrogen-oxidizing bacteria (NOB), and soil microbial biomass were compared with patch-derived volume along a 110-year longleaf pine (*Pinus palustris* Mill.) chronosequence for identifying a trajectory and ecological benchmark during forest restoration. Net nitrogen mineralization rates decreased significantly in the maturing-aged, pine patches, driven by a larger drop in net nitrification. Net nitrification and abundance of NOB were higher in young pine patches compared to soils from the maturing (86–110 years) pine patches. Gross nitrate fluxes followed the nonfungal portion of the soil microbial biomass along the chronosequence, declining in 64-year-old pine patches. Microbial biomass peaked in patches 17–34 years of age, but significantly declined in the older patches. Fungal biomass leveled off without decline. Ammonium was the major source of nitrogen within the maturing pine patches as well as the wetland patches, indicating that ammonium maintains longleaf pine during growth-limiting conditions. Nitrate dominated during rapid tree growth, optimally in mesic conditions. The relative amounts of available ammonium to nitrate can be used to model nitrogen cycling in facultative-wetland pine forests of the coastal United States as soils alternate between wet and mesic conditions. A key restoration benchmark occurred after 86 years of pine development when pine patch growth rates slowed, with lower numbers of NOB, when the nonfungal biomass leveled off, and net nitrification rates are at a minimum, during pine maturation.

Key words: chronosequence, ergosterol, microbial biomass, nitrogen mineralization, nitrogen-oxidizing bacteria

Conceptual Implications

- The restoration of forest ecosystems is dependent on robust forest growth and the availability of nutrients to produce that growth.
- Soil microbes are the mediators of this interdependence and can be measured by mass or abundance to verify the level of soil nitrogen cycling during a given stage of forest development.
- Soil nitrate fuels rapid forest growth in mesic environments, but ammonium maintains forest health during stand maturation or when anaerobic conditions exist in wet soils.
- This interaction has been evaluated by the relative supply of ammonium (NH_4^+) to nitrate (NO_3^-), soil microbial biomass, the abundance of nitrifying bacteria, and changes in patch volume for the purpose of determining a restoration benchmark at a certain point on the restoration trajectory.

Introduction

Ecological restoration requires repairing the functions within soils as well as the structure of forest ecosystems (Johnston & Crossley 2002; Harris 2003; Heneghan et al. 2008). For longleaf pine (*Pinus palustris* Mill) ecosystems to be resilient (self-recovering) to degradation, they need to have a vigorous cycling of nutrients for biomass accumulation (Vitousek & Reiners 1975; Holling 1996; Johnston & Crossley 2002; McCaskill 2008). Ecosystem resilience is considered as a major goal of restoration efforts and has been described in terms of nitrogen retention or the ability of an ecosystem to limit nitrogen loss (Odum 1969; Vitousek & Reiners 1975; Pandey et al. 2009; Shade et al. 2012). Ecosystems are considered "leaky" when nitrate (NO_3^-) is found in higher concentrations or "tighter" when the less-mobile ammonium (NH_4^+) is the predominant form of inorganic nitrogen (Vitousek & Reiners 1975; Huygens et al. 2007). The steady-state condition of forest succession has been described as the time period when an ecosystem's nitrogen supply is in balance with forest growth (Odum

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1969; Oliver 1981; Oliver & Larson 1996). Vitousek and Reiners (1975) concluded that nitrogen retention in an ecosystem actually "mirrors" biomass accumulation during stand development. They further concluded the "tightest" period of nitrogen retention occurred when the availability of inorganic nitrogen was brought into short supply by heavy vegetative competition during the stem exclusion stage. But the retention of nitrogen in forests is impacted by its inorganic form as well as its availability under specific successional conditions. The moisture content of soils must be factored in when evaluating the relative supply of ammonium to nitrate (Gijsman 1990; Gijsman & Van Noordwijk 1991; Everett et al. 2010).

In restoration ecology literature, there are many references to restoring the soils of ecosystems (Hobbs & Harris 2001; Johnston & Crossley 2002; Harris 2003; Banning et al. 2011; McCaskill & Jose 2012; Piche & Kelting 2015). The relationship between changes in stand growth rates and the level of inorganic nitrogen turnover can be used to identify a steady-state restoration threshold when stand growth rates have slowed, and when the available supply of ammonium exceeds nitrate in maturing forests (Vitousek & Reiners 1975; Nave et al. 2014). Combining the pattern of forest development with the process of nutrient cycling is essential in identifying any meaningful restoration threshold (Johnston & Crossley 2002; Harris 2003; Bestelmeyer 2006; Shade et al. 2012). Research results from restoration projects conducted within pine forests of the southeastern United States linking stand productivity with soil nitrogen cycling have mostly focused on the conversion of loblolly (*Pinus taeda*) or slash (*Pinus elliotti*) pine plantations to longleaf pine savannas (Van Lear et al. 2005; McCaskill 2008; Jose et al. 2010; McCaskill & Jose 2012; Foote et al. 2015). Restoration research conducted in other ecotypes is also found in the literature (Vance & Entry 2000; Stanturf et al. 2001; Banning et al. 2011; Piche & Kelting 2015). However, studies concerning soil nitrogen dynamics in facultative-wetland habitats, some of the most threatened ecosystems in the coastal portions of the southern United States, are few (Harms et al. 1998; McCaskill 2008; McCaskill & Jose 2012). An earlier longleaf pine study found that nitrogen mineralization rates declined as the moisture gradient increased, assessing soils from xeric sandhills through mesic flatwoods to wet pine savannas, but the levels of tree biomass, total soil carbon and nitrogen, and microbial biomass exhibited reverse trends (Wilson et al. 2002).

This is a great example of the interactions, which exist among forest growth, nitrogen cycling, and differences within microbial communities, to soil moisture (Cookson et al. 2007; Berkowitz & White 2013; Carrillo et al. 2016).

The purpose of this work was to identify a restoration trajectory and a benchmark (restored condition point) from measured reference conditions within coastal wet longleaf pine ecosystems (Hobbs & Harris 2001; Van Lear et al. 2005; Bestelmeyer 2006). Our restoration study centered on identifying the links between changes in wood accumulation rates and soil nitrogen transformations along a 110-year patch-derived longleaf pine chronosequence to discover this restoration trajectory and a point of restored condition (benchmark) along the trajectory (Hobbs & Harris 2001; Johnston & Crossley 2002; Bestelmeyer 2006; Shade et al. 2012; Piche & Kelting 2015). This was accomplished through the analysis of soil biogeochemical data (net ammonification [NH_4^+] and nitrification [NO_3^-], abundances of ammonium-oxidizing bacteria [AOB] and nitrite-oxidizing bacteria [NOB], soil fungal [C_{FB}] and microbial [C_{MB}] biomass [carbon]), collected from 36 longleaf pine reference patches and six control (untreated) plots from the restoration site (Foster & Tilman 2000; Aravena et al. 2002; McCaskill 2008; Jose et al. 2010; Walker et al. 2010; McCaskill & Jose 2012). These analyses were used for identifying restoration benchmarks (restored conditions) for coastal wet longleaf pine flatwoods (Christensen & MacAller 1985; Johnston & Crossley 2002; Chapman et al. 2003; Harris 2003; McCaskill & Jose 2012). There was a secondary desire to examine soil nitrogen cycling by the relative supply of available ammonium to nitrate along a soil moisture gradient based upon our previously classified mesic flatwood, wet flatwood, and wet savanna pine patches for comparison (Gijsman 1990; Gijsman & Van Noordwijk 1991; Everett et al. 2010; McCaskill & Jose 2012; Berkowitz & White 2013; Liu et al. 2014).

We hypothesized high rates of net nitrification and microbial biomass accumulation in response to rapid pine growth during the early-age (6–34 years) stage of pine patch development. We also predicted that these biogeochemical processes would decrease at some point after the mid-age stage (60–71 years) and reach an asymptote as pine growth rates slowed during patch maturation. This soil biogeochemical asymptote can be considered as a benchmark along the restoration trajectory for longleaf pine ecosystems when soil productivity is considered to be resilient to degradation (Johnston & Crossley 2002; Bestelmeyer 2006; Shade et al. 2012; Piche & Kelting 2015). We also expected that these postulations would be impacted by the soil moisture conditions of the individual pine patches across our reference sites (McCaskill & Jose 2012). We still expect to observe smaller abundances of NOB in response to a lower demand for nitrate within the maturing (86–110 years) pine patches compared to our young fast-growing (6–17 years) pine patches. We will attempt to identify a forest restoration benchmark based upon net nitrification levels, AOB/NOB abundances, nonfungal microbial biomass, and changes in forest patch volume along the chronosequence (restoration trajectory; McCaskill 2008; McCaskill & Jose 2012).

Methods

Wet longleaf pine ecosystems are highly disturbed and fragmented along Florida's Gulf coast due to hurricanes, fire, wind, plantation forestry, and urbanization (Myers & Van Lear 1998; Palik et al. 2002; Stanturf et al. 2007; Domec et al. 2015). As a result, these forests are generally found as distinct single or two-aged patches (cohorts) forming a mosaic along the Gulf coast between the western Panhandle of Florida and the central peninsula near Tampa Bay (Palik et al. 2002; Gagnon et al. 2004; Stanturf et al. 2007; McCaskill & Jose 2012). A patch-derived chronosequence was established to fit this landscape based upon three reference sites and a restoration location

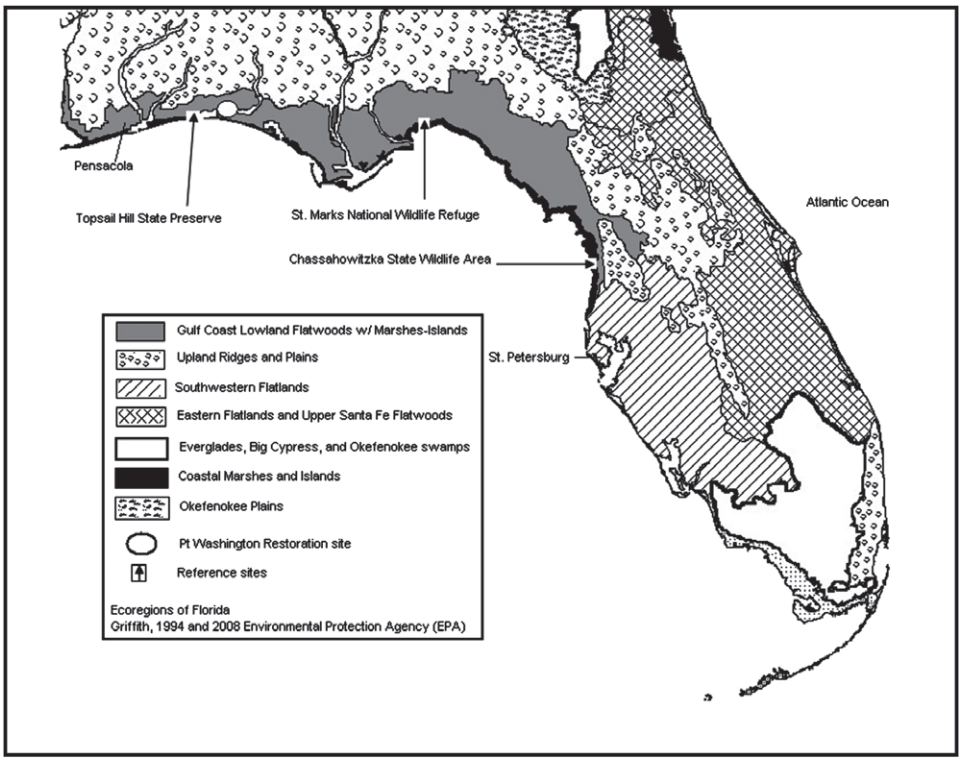

Figure 1. Name and location of reference and restoration sites on the Gulf coast of Florida, USA (modified from McCaskill & Jose 2012).

west of Panama City (Pickett 1989; Johnson & Miyanishi 2008; Myster & Malahy 2008; Walker et al. 2010). The chronosequence was a result of 36 measured 400 m^2 pine patches stratified into 26 differently aged cohorts, and grouped into five distinct age-intervals for analysis (McCaskill 2008; McCaskill & Jose 2012). Six untreated control plots from the restoration site were combined with the 6-year-old pine patches from the reference locations (McCaskill & Jose 2012). As most of this landscape is very close to sea level, the dataset was used to ecologically classify each of the longleaf pine patches as either mesic flatwood, wet flatwood, or wet savanna. These classifications were identified by the forest structure, the presence of obligate wetland, facultative, or obligate upland plants, and the hydric soil conditions, assessed for each patch (Tiner 1999; McCaskill & Jose 2012).

Gulf Coast Reference Sites

The three representative locations were inventoried within the Gulf coast of Florida (Fig. 1; Griffith et al. 2008; McCaskill & Jose 2012) and selected as reference sites for evaluating the Point Washington restoration site near Panama City, Florida. All three reference sites contain similar plant communities to the restoration site; composed of *Andropogon virginicus*, a smaller component of *Aristida stricta*, and a group of shrub species highlighted by *Ilex glabra*, *Serenoa repens*, *Quercus pumila*, and *Gaylussacia frondosa*. Wetter areas had a greater presence of *Lachnanthes caroliniana*, *Cliftonia monophylla*, *Nyssa sylvatica* var. biflora, *Cyperus*, *Scleria*, *Xyris*, and *Lachnocaulon* (Reinman 1985; Peet & Allard 1993; White et al. 2000; Spencer 2004; McCaskill 2008; McCaskill & Jose 2012), as well as common soils as the restoration site (Table 1; Hyde et al. 1977; Overing & Watts 1989; Allen 1991; McCaskill & Jose 2012; Swanhart et al. 2013).

The Chassahowitzka Wildlife Management Area (28°78′47″N, 82°34′26″W) in Hernando County, Florida contains approximately 12,140 ha of forests and has soils within the Myakka fine sands and Basinger fine sands soil series (Table 1; Hyde et al. 1977). The St. Marks National Wildlife Refuge (30°6′18″N, 85°11′7″W) in Wakulla County, Florida consists of 25,900 ha of forests and marshes. Their soils have parent material derived from marine deposits and are dominated by the Leon and Scranton soil series (Table 1; Allen 1991). The Topsail Hill State Park (30°22′15″N, 86°16′20″W) in Walton County, Florida contains 610 ha of older longleaf pine and its soils are included in the Leon and Pickney sand soil series with their parent material also being derived from marine deposits (Table 1; Overing & Watts 1989).

Point Washington Restoration Site

The Point Washington restoration site (30°20′N, 86°4′W) in Walton County, Florida was a 4 ha, 26-year-old wet slash pine plantation with an average diameter at breast height (dbh) of 19.1 cm and an average basal area of 7.85 m^2/ha, as measured in 2001. The site was harvested, burned, and planted with longleaf pine between fall 2001 and spring 2002 (Jose et al. 2010).

Table 1. Soil and stand properties between reference and restoration sites (McCaskill & Jose 2012). *Means followed by the same lower case letters are not significantly different ($\alpha = 0.05$).

Location	*Soil Great Group*	*Soil Texture (Top 10 cm)*	*Moisture Regime*	*Temperature Regime*	*Drainage Class*
Chassahowitzka Wildlife Management Area	Psammaquent	Sandy	Aquic	Hyperthermic	Very poorly drained
	Alaquod	Sandy	Aquic	Hyperthermic	Poorly drained
St. Marks National Wildlife Refuge	Psammaquent	Sandy	Aquic	Thermic	Very poorly drained
	Alaquod	Sandy	Aquic	Thermic	Poorly drained
Topsail Hill State Preserve	Humaquept	Sandy	Aquic	Thermic	Very poorly drained
	Alaquod	Sandy	Aquic	Thermic	Poorly drained
Point Washington restoration site	Psammaquent	Sandy	Aquic	Thermic	Very poorly
	Alaquod	Sandy	Aquic	Thermic	Poorly drained

Stand Basal Area and Soil Biochemical Properties (Mean Values)*

Drainage class	*Stand basal area (m^2/ha)*	*Soil pH [H+]*	*Soil organic matter content (%)*	*Net nitrogen mineralization (mg N kg^{-1} soil $month^{-1}$)*	*Microbial biomass carbon (mg carbon/kg soil)*
Very poorly drained	6.5a (± 1.5)	4.39a (± 0.07)	3.1a (± 0.43)	11.6a (± 2.9)	374.3a (± 70.3)
Poorly drained	8.3a (± 1.6)	4.45a (± 0.08)	1.8b (± 0.21)	9.9a (± 2.2)	356.1a (± 62.8)

The soils belong in the Leon series having parent material derived from marine deposits (Table 1; Overing & Watts 1989; McCaskill & Jose 2012). The adjacent area is approximately 20 ha of mixed slash and longleaf pine surrounding a large cypress dome, and part of the greater 6,800 ha Point Washington State Forest.

Longleaf Pine Development Stages

We previously identified three general stages of longleaf pine development as early-age, mid-age, and maturing stages, then stratified them into five distinct age-intervals (Oliver 1981; Oliver & Larson 1996; McCaskill & Jose 2012). The forest and soil data were then analyzed for differences in forest structure and soil biogeochemistry among the differently aged pine patches. As mentioned earlier in this Methods section, the dataset was then used to ecologically classify each of the longleaf pine patches as either: mesic flatwood, wet flatwood, or wet savanna (Tiner 1999; McCaskill & Jose 2012).

The early-age stage of pine development (6–34 years) had at least 70% of the stocking as a combination of seedlings/saplings, poles, and scattered small sawlog-sized trees. The young-age interval (6–10 years) within this stage had 70% of its stocking as seedlings/saplings and a few pole trees. Pine patches between 17 and 34 years had more poles and small sawlog-sized trees (10–30 cm dbh). The mid-age stage (36–71 years) patches had 70% of its stocking as a mixture of small- and large sawlog-sized trees (31–45 cm dbh). Patches between 36 and 52 years had greater numbers of the smaller sawlog trees. Those patches between 60 and 71 years had more large sawlog-sized trees. The maturing stage (86–110 years) pine patches were dominated with large sawlog-sized trees and prominent tree gaps in their landscapes (Gagnon et al. 2004).

Each development stage was determined by measuring tree height and diameter on all trees greater than 10 cm dbh. Saplings and seedlings were measured for dbh or root collar. Saplings have a dbh of 2.54 cm or greater, whereas seedlings are shorter than 1.37 m and have their diameters measured at the root collar. Patch volume (m^3/ha) was calculated from these data. At least 30% of the representative trees within each patch were cored at breast height to determine patch age (McCaskill & Jose 2012).

Sampling Design

At each reference location, three 1-hectare blocks were established representing each of early-, mid-age, and maturing stages of longleaf pine development. Each block was subdivided into four randomly placed 400 m^2 patches where forest structure and age were determined Gagnon et al. 2004; McCaskill 2008; McCaskill & Jose 2012). Soils were sampled within four randomly placed 1 m^2 quadrats located in each 400 m^2 patch, using a graduated trowel and taken from the top 10 cm of the surface minus the O horizon. Collected reference data were combined with field data sampled from the control (untreated) plots containing 6-year old pines, located on the Point Washington State Forest restoration site (McCaskill & Jose 2012).

Soil Sampling and Preparation

Forty-eight soil samples (≥500 g) were collected in the upper 10 cm of the "A" horizon from each reference location and the six control (untreated) plots on the restoration site during the 2005–2006 growing seasons and immediately stored at 4°C until analysis. Soil tests were performed on all 192 soil samples ($n = 48$) incorporating three replications. Subsamples (20 g) were analyzed for soil pH by prepared slurries using a soil-to-water ratio of 1:2 (McLean 1982), percent soil organic matter (SOM) content by the Walkley–Black method (Walkley 1947) utilizing chromic acid to measure the oxidizable organic carbon in a soil, and a soil subsample was sieved through 2-mm mesh and dried for 3 days (105°C) to determine gravimetric moisture content (Black 1965).

In Situ Net Nitrogen Mineralization

Net nitrogen mineralization (N_{min}) was determined by the buried bag technique as described by Eno (1960). In general, a (350 g, 5–15 cm depth) soil sample in a polyethylene 30 µm bag was taken to the soil lab for immediate analysis while a second bag was buried (15 cm depth) in situ for incubation during the months of April and August, 2005; a process repeated for 2006 (Isaac & Timmer 2007). Mineral nitrogen was extracted with 60 mL 2 N KCl from 20 g of soil according to Keeney and Nelson (1982). The samples were analyzed for ammonium (NH_4^+) and nitrate (NO_3^-) using a Seal AutoAnalyzer II with a continuous segmented flow.

Bacterial Abundance

Enumeration of NOB was determined by the most probable numbers (MPN) method for AOB and NOB using a five-tube dilution (Schmidt & Belser 1982). The AOB were incubated in a medium of diammonium sulfate, and the NOB were incubated in potassium nitrite. The final enumeration of bacteria was obtained after 16 weeks of incubation. The presence of AOB was determined by a pH indicator (phenol orange) and NOB by a nitrate test reagent of diphenylamine in sulfuric acid solution (Schmidt & Belser 1982). This procedure was only conducted with soil samples taken from young-aged (6–17 years) and maturing (86–110 years) pine patches from a mesic St. Marks NWR reference site and a wet savanna site located at the Topsail Hill Preserve reference location (McCaskill & Jose 2012).

Microbial/Fungal Biomass

Soil microbial biomass carbon (C_{MB}) was determined by chloroform fumigation–extraction according to Vance et al. (1987). A 0.05 M K_2SO_4 extractant was used to remove carbon from the control and fumigated soil samples taken from low-pH soils (Haney et al. 2001). Samples were shaken for 1 hour and centrifuged (6,000 rpm) for 15 minutes before filtering the supernatant through no. 42 Whatman filter paper. Total organic carbon (TOC) was determined on a Shimadzu TOC-VCSH analyzer according to Vance and Entry (2000). Microbial biomass carbon was calculated as ([fumigated TOC – control TOC]/0.51)/(soil dry weight) = mg C_{MB}/kg soil (Joergensen 1996).

Soil fungal biomass carbon (C_{FB}) was determined by the extraction of ergosterol from soil samples (Gong et al. 2001). Each sample had ergosterol extracted with methanol (0°C) by shaking for 1 hour at 360 revolutions/minute and centrifuged at 11,000 rpm for 20 minutes. The microfiltered supernatant (1.5 mL) was measured by high performance liquid chromatography (HPLC) at 282 nm (Beckman Coulter System Gold HPLC) and expressed in micrograms (µg) ergosterol per g soil. Multiplying 3.65 µg ergosterol/mg soil by 220 converts the amount to fungal biomass (mg carbon/g soil; Montgomery et al. 2000).

We subtracted the fungal component from the overall microbial biomass with the assumption that the remaining amount (C_{bac}) is closer to the amount of microbial biomass directly involved in aerobic nitrification.

Statistical Analysis

A three-stage balanced nested design was used to integrate the indicators measured at different scales and between sites. There was a minimum of four replicates for stand data and four replicates for the soil data (McCaskill & Jose 2012). Mean values per patch for tree diameter, tree height, tree age, tree density, as well as patch volume, were analyzed with net ammonium-to-nitrate levels, AOB/NOB abundances, and nonfungal microbial biomass as fixed effects through a mixed model restricted maximum likelihood (REML) analysis producing *F*-ratios (PROC Mixed; SAS 2008). The random effects were the age-intervals, 400 m^2 patches, 1 m^2 vegetation quadrats, the location of reference sites, and the six control plots located within the experimental blocks at the restoration site. Hypothesis testing for differences between age-interval means was accomplished by using Tukey's *t*-test with an α of 0.05 and a two-tailed confidence interval. Trends between variables were obtained from nonlinear regression (Proc NLIN, SAS 2008). The $\log_{(x)}$ data transformations were applied where necessary to stabilize variances prior to analysis, then followed by nonlinear regression (Fortin & Dale 2005; Proc NLIN, SAS 2008).

Results

Much of the variability between NH_4^+ and NO_3^- for the nitrogen mineralization ratios measured in the patches across the chronosequence is due to almost 45% of the patches containing wetland conditions (Tiner 1999; McCaskill & Jose 2012). The identified facultative-wetland patches (Fig. 4) by age in years are: 8, 9, 10, 24, 25, 28, 34, 36, 40, 52, 62, and 71 (McCaskill & Jose 2012). Some of the patch ages not listed above had only a portion of their areas under facultative-wetland conditions.

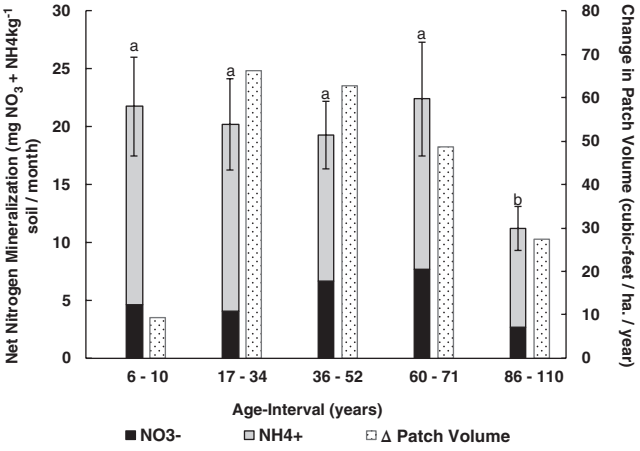

Figure 2. Net nitrogen mineralization rates $NH_4^+ + NO_3^-$ (mg N kg^{-1} soil $month^{-1}$) with changes in the rate of patch volume accumulation (cubic-feet per hectare/year) sampled from pine patches at different age-intervals of pine development, across the chronosequence. Error bars represent ±95% confidence intervals around the estimated mean. Different letters indicate statistically significant differences ($p < 0.05$).

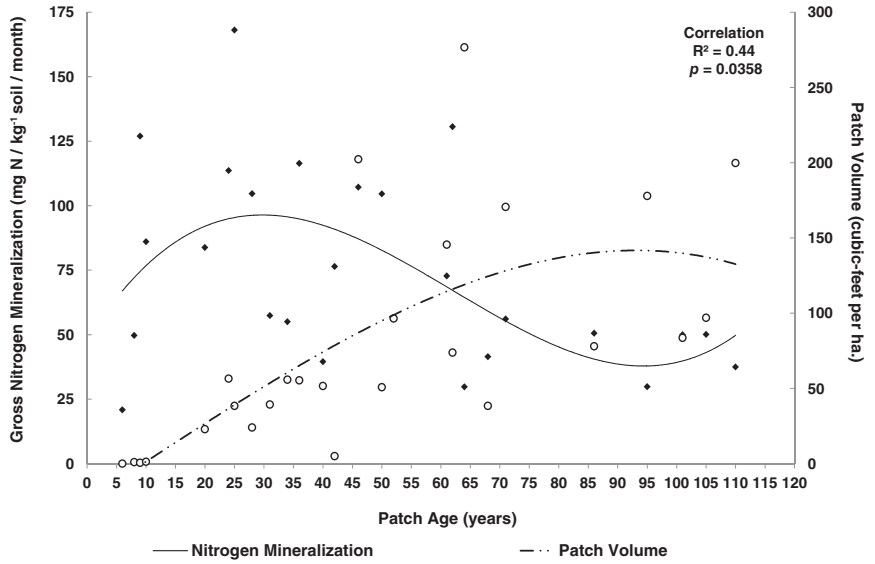

Figure 3. Gross nitrogen mineralization (N_{min}) in mg nitrogen kg^{-1} soil $month^{-1}$, plotted with patch volume (cubic-feet per hectare); sampled from differently aged pine patches, across the chronosequence. Correlation (Pearson) is between N_{min} and patch volume with patch age as the weight variable.

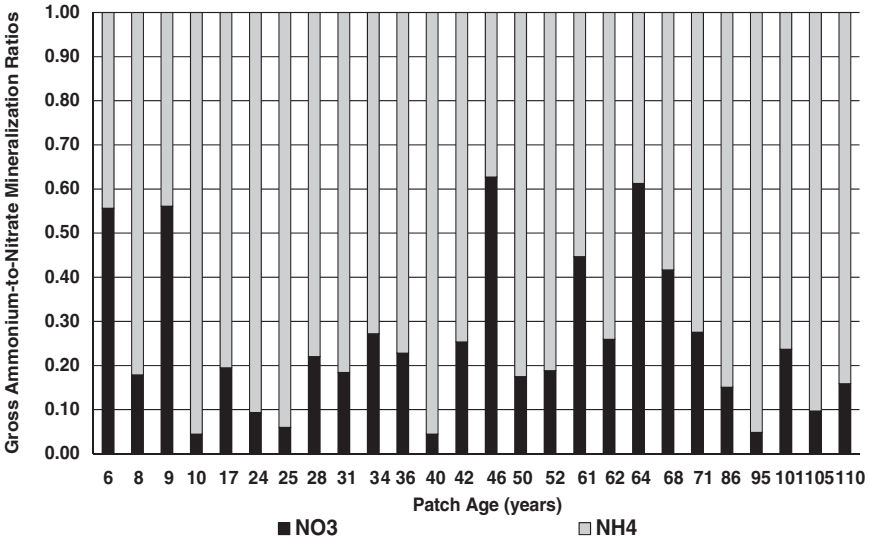

Figure 4. Net nitrogen mineralization rates as the ratio of available ammonium (NH_4^+) to available nitrate (NO_3^-) (mg N kg^{-1} soil $month^{-1}$) sampled from differently aged pine patches, across the chronosequence.

Temporal Patterns

Wood accumulation rates increased with patch age, only decreasing in pine patches 86 years old or greater (Fig. 2; mixed model REML t =31.7, p = <0.0001; SAS 2008). Net nitrogen mineralization rates were highest in the 60–71-year-old patches, decreasing to their lowest levels within the 86–110-year-old patches (mixed model REML t =6.49, p = <0.0001), likely driven by net nitrification fluxes (mixed model REML t =13.04, p = <0.0001), which also had their highest levels in the 60–71-year-old patches, before declining (Fig. 2). The magnitude and variation for nitrogen mineralization fluxes diminished in pine patches 64 years old and older, as patch volume plateaued (Fig. 3; mixed model REML t =11.90, p = <0.0001; Proc CORR, Pearson R = 0.44, p = <0.0358; Odum 1969; Vitousek & Reiners 1975). Gross nitrogen mineralization measured as the ratio of available ammonium to nitrate was dominated by ammonium (Proc CORR, Pearson R = 0.91, p = <0.0001), with nitrification fluxes representing greater than 25% of the nitrogen mineralization levels within 11 of the differently aged pine patches across the chronosequence (Fig. 4). Nitrate fluxes and AOB/NOB numbers were significantly higher (Proc T Test t =2.35, p = 0.0279) in the young (6–17 years) patches compared to the maturing (100 years old) pine patches (Table 2). Increases or decreases in the nonfungal portion (C_{bac}) of the microbial biomass were

Table 2. MPN enumerations of nitrogen-oxidizing bacteria, ammonification, and nitrification in young and maturing longleaf pine forest soils. All MPN values are expressed in units of MPN per gram (wet weight) of 0–10-cm soil and are averages of three replicates. Lower and upper limits in parentheses reflect 95% confidence intervals. Net N_{min} rates are based upon monthly incubations.

			Enumerations (MPN/g)		*Net Nitrogen Mineralization*		
Site	*Age-interval*	*Patch age (yr)*	*Ammonium oxidizers*	*Nitrite oxidizers*	*Net N_{min} (mg kg^{-1} soil yr^{-1})*	*Net NH_4^+ (mg kg^{-1} soil yr^{-1})*	*Net NO_3^- (mg kg^{-1} soil yr^{-1})*
St. Marks NWR	Early	6	1.4690×10^4 (0.278, 6.318)	0.4273×10^3 (0.103, 1.385)	24.63	10.93	13.69
St. Marks NWR	Mature	100	0.0427×10^4 (0.103, 1.385)	0.0040×10^3 (0.005, 0.123)	42.29	37.88	4.41
Topsail Hill State Park	Early	17	0.0240×10^4 (0.047, 0.965)	0.4273×10^3 (0.103, 1.385)	170.91	154.09	16.82
Topsail Hill State Park	Mature	100	0.0004×10^4 (0.005, 0.123)	0.0036×10^3 (0.005, 0.123)	56.38	42.53	13.85

followed by higher or lower nitrate fluxes, respectively (Fig. 5; Proc NLIN log F-value = 8.52, $p = <0.0041$; Proc CORR, Pearson $R = 0.39$, $p = <0.0465$). The nonfungal portion of the microbial biomass carbon (C_{bac}) accumulated with patch age, peaking in pine patches 17–35 years old (Fig. 6; mixed model REML t =3.93, $p = <0.0005$). The nonfungal portion of the microbial biomass carbon (C_{bac}) significantly declined in pine patches 86–110 years of age (Fig. 6; mixed model REML t =3.44, $p = <0.0022$). Fungal biomass also increased with patch age, leveling off in pine patches 60–71 years old or greater (Fig. 6; mixed model REML t =6.58, $p = <0.0001$; SAS 2008).

Spatial Patterns

The abundance of AOB measured at the mesic St. Marks (6-year-old pines) patches were significantly larger (14,690 g^{-1} soil) compared to the corresponding AOB numbers measured within the wet (17-year-old pines) patches at Topsail Hill State Park (240 g^{-1} soil), resulting in significantly lower net ammonification rates, 11 mg NH_4^+ kg^{-1} soil $month^{-1}$ versus 154 mg NH_4^+ kg^{-1} soil $month^{-1}$ (Table 2). The St. Marks site also had significantly greater abundance of AOB from its mesic maturing (100-year-old pines) patches (427 g^{-1} soil vs. 4.0 g^{-1} soil) compared to the wet (100-year-old pines) patches from the Topsail Hill site, but there was no significant difference in net ammonification rates among locations (Table 2). The abundance of NOB showed no significant differences between sites, regardless of pine patch age or hydric soil conditions. Net nitrification fluxes from the mesic 100-year-old pine patches within St. Marks were lower (4.4 mg vs. 13.85 mg NO_3^- kg^{-1} soil $month^{-1}$) compared to the wet 100-year-old pine patches at Topsail Hill preserve (Table 2). Net ammonification had no significant differences in the mature patches.

Discussion

Any significant differences in the abundance of AOB, nitrogen turnover rates, or the levels of soil microbial biomass carbon can be directly attributed to changes in stand volume over time, and not solely differences in soil moisture content (Zak et al. 1990; Dickens et al. 2015). Wood accumulation rates slowed within the maturing pine patches as trees shifted from height growth and radial increment to only slow radial accumulations (Oliver & Larson 1996). These lower wood accumulation rates resulted in the lower net nitrification fluxes and the lower abundance of NOB as the demand for nitrate decreased (Vitousek & Reiners 1975; Rosswall 1976; Christensen & MacAller 1985; Zak 2014). An earlier study found similar results from researchers studying succession in a Norway spruce (*Picea abies*) forest, where they found large numbers of NOB (AOB + NOB) in sites recently harvested, but detected small numbers (<10 g^{-1} soil) in mature, less-disturbed forests (Paavolainen & Smolander 1998). The interaction between the nonfungal microbial biomass (C_{bac}) and gross nitrification rates over the chronosequence is another illustration of microbes responding to changes in their environment while satisfying the demand for nitrate needed for stand growth (Morris & Boerner 1998; Chapman et al. 2003; Cookson et al. 2007; Walker et al. 2010; Banning et al. 2011). Similar relationships between bacterial abundance, microbial biomass, and nitrification have been found in other studies where microbes, nitrate transformation, forest restoration, and soil water interact across a landscape (Pandey et al. 2009; Banning et al. 2011). They found the fungal community to be less responsive to successional change than the bacteria. It is worth noting that when we observed increases in the bacteria biomass within the maturing-aged pine patches, it did not produce the same response in nitrification fluxes realized in the younger-aged pine patches. Also, while the nonfungal biomass decreased in the maturing-aged pine patches compared to the younger-aged patches, fungal biomass levels plateaued. Our results on nitrification, abundances of nitrifying bacteria, and changes in nonfungal biomass illustrate a demand for nitrate during rapid stand growth in the young pine patches that becomes coupled to an increased capacity for microbial-mediated transformations, which is then followed by the consumption of ammonium through aerobic oxidation (Richards 1987; Sylvia et al. 1998). Eventually stand growth

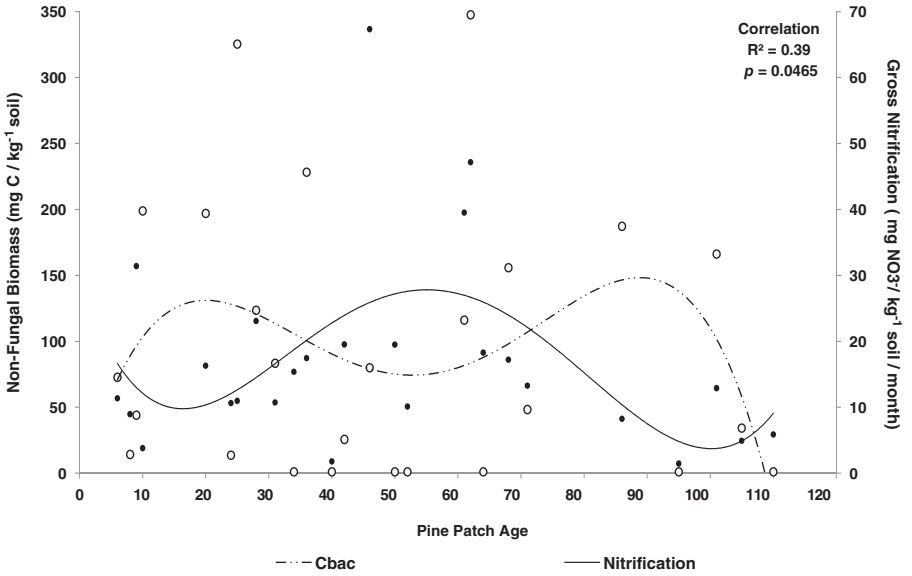

Figure 5. Nonfungal (C_{bac}) microbial biomass (mg carbon/kg soil) plotted with gross nitrification (mg NO_3^- kg^{-1} soil $month^{-1}$) sampled from differently aged pine patches, across the chronosequence. Correlation (Pearson) is between C_{bac} and nitrification with patch age as the weight variable.

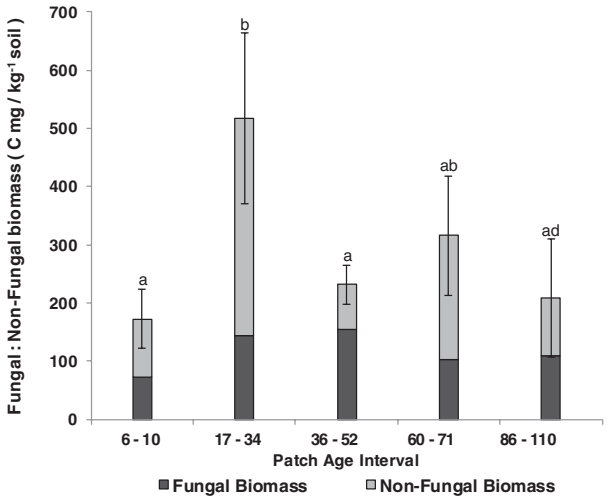

Figure 6. Soil fungal (C_{FB}) and nonfungal (C_{bac}) microbial (C_{MB}) biomass carbon (mg carbon/kg soil) sampled from pine patches at different age-intervals of pine development, across the chronosequence. Error bars represent ±95% confidence intervals around the estimated mean. Different letters indicate statistically significant differences ($p < 0.05$).

slows in the mature-aged patches, and with a smaller abundance of nitrifying bacteria, nitrification drops.

Before there is a discussion about our results concerning the interaction between ammonium and nitrate within facultative-wetland landscapes, we must first distinguish between ammonification and bacteria-mediated aerobic nitrification, the two nitrogen transformation processes of mineralization taking place in forests during restoration, but whose origins along Earth's evolutionary timeline started from two distinct time periods (Mancinelli & McKay 1988; Johnston & Crossley 2002; Canfield et al. 2010; Isobe & Ohte 2014; Piche & Kelting 2015). Ammonification was one of the earliest chemical transformations to evolve on Earth, followed by anaerobic bacteria-mediated denitrification, and then as the atmosphere became oxygenated, aerobic nitrification evolved (Mancinelli & McKay 1988; Canfield et al. 2010). Also, in ecology, we value species richness and functional diversity as ways to measure an ecosystem's resilience (Holling 1996; Hobbs & Harris 2001). Ammonification is a species-rich transformation whereas aerobic nitrification is a species-poor process (Isobe & Ohte 2014). There are ancient oligotrophic ammonia-oxidizing archaea whom are able to function in very low concentrations of ammonia (NH_3^+) or in acidic soil conditions, but these dramatically smaller microbes reproduce very slowly (Hatzenpichler 2012; Prosser & Nicol 2012). In contrast, many species of heterotrophic bacteria (e.g. *Pseudomonas*), actinomycetes, and the fungi are the active mediators during the production of ammonium from a large pool of organic nitrogen (Sylvia et al. 1998; Inselsbacher & Nasholm 2012; Isobe & Ohte 2014). Ammonification is also a facultative-wetland process, readily occurring in both anaerobic and aerobic conditions, making the range of environments for this transformation, enormous. Most species of chemoautotrophic bacteria convert nitrite to nitrate under mostly aerobic conditions, restricting drastically the range of environments (Richards 1987; Sylvia et al. 1998). The major problem with the ancient ammonia-oxidizing archaea is their inhibition in ammonium-rich substrates (Prosser & Nicol 2012; Stahl & de la Torre 2012). Ammonification has the advantage of being readily adaptable to facultative-wetland conditions while producing ammonia or ammonium at non-limiting levels. Ammonification has been sustaining life long before bacteria-mediated nitrification came to prominence as an important transformation process within the aerated terrestrial environments (Mancinelli & McKay 1988; Canfield et al. 2010).

The results of the ratio of ammonium-to-nitrate fluxes across each of the differently aged pine patches showed great variability, but it is clear from the results that ammonification dominated this landscape. Many of the very low nitrification rates observed across the chronosequence correspond to those patches identified as having wetland conditions. We identified close to 45% of the pine patches having facultative-wetland conditions, not including those unlisted patch ages where a portion of their patches contained wetland characteristics (Tiner 1999; McCaskill & Jose 2012). As the supply of available ammonium (NH_4) was higher than nitrate (NO_3) across the chronosequence, a nitrogen-conserving (tighter) condition existed within the facultative-wetland pine patches (Huygens et al. 2007; Berkowitz & White 2013). The conservation of nitrogen is tied to ammonium being the less-mobile form of inorganic nitrogen within wet environments, whereas nitrate could be lost through leaching or easily converted to N_2 gas (Huygens et al. 2007; Pandey et al. 2009). There was also an indication of a nitrogen-conserving condition within the maturing patches as smaller numbers of NOB and lower net nitrification fluxes were detected compared to the fast-growing early-aged patches. This is an important finding for facultative-wetland forests where seasonal flooding impacts both stand growth and the cycling of nitrogen during succession (Kreuzwieser et al. 2002; Liu et al. 2014).

Restoration monitoring requires the use of process-oriented indicators as well as ecosystem measurements of structure and composition in order to determine the long-term success of projects (Falk 2006; Herrick et al. 2006; McCaskill 2008). Our results provide some insights into how properties of soil biogeochemistry can be used as indicators to evaluate restoration projects by separating out different environmental conditions along a restoration trajectory or among sites (Vance & Entry 2000; Chapman et al. 2003; Harris 2003; McCaskill & Jose 2012). The abundance of nitrifying bacteria, nitrogen mineralization rates, the levels of soil microbial biomass carbon, and changes in stand volume were successful in detecting changes along a restoration trajectory. We also found AOB to be sensitive at detecting differences in environmental conditions among reference sites, whereas the limited abundance of NOB were not (Mota et al. 2005; Everett et al. 2010). The abundance of NOB detected differences between young-aged and mature-aged pine patches, but were too small to detect any difference in environmental conditions between sites as indicated by similar NOB numbers at the two sites, regardless of patch age (Gijsman 1990; Paavolainen & Smolander 1998; Mota et al. 2005). Much of Topsail Hill State park had a longer seasonal period of high water (hydroperiod) than the St. Marks NWR, resulting in lower numbers of AOB than at the corresponding sites for the St. Marks site (Kreuzwieser et al. 2002; McCaskill & Jose 2012; Liu et al. 2014). The difference in NOB explains why ammonification fluxes had reverse trends between the two sites. Some spatial heterogeneity pertaining to tree stocking was observed in the pine patches found within the 61–71-year age-interval, probably confounding the interaction between soil moisture and the pattern for nitrogen cycling for that particular age-interval of the chronosequence. But, most of the biogeochemical differences between patches can be attributed to the presence of facultative-wetland conditions.

Nonfungal and fungal biomass accumulated with patch age, but nonfungal biomass was more responsive to differences among the patches or reference sites.

Ammonium dominated inorganic nitrogen fluxes in the maturing-aged pine patches as well as in patches identified as having facultative-wetland conditions. Both of these situations are where you would expect to see limited tree growth rates, indicating that ammonium maintains longleaf pine during stand maturation or during flooding. Nitrate serves primarily as the fuel for rapid tree growth during stand development, optimally in mesic conditions.

A key restoration benchmark for these longleaf pine patches is observed when the wood accumulation rates of our patches have declined during maturation, reducing the demand for nitrate. This benchmark is further indicated by the soil microbial biomass leveling off, by lower numbers of NOB, and when the net nitrification rates are at a minimum. These conditions were met in spite of the fact that 45% of the pine patches contained facultative-wetland conditions.

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