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ARTICLE

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Soil microbiomes of hardwood- versus pine-dominated stands: Linkage with overstory species

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Abstract

Biodiversity in forest ecosystems is paradoxical. Whereas their most apparent component—the woody overstory—is the least diverse with respect to numbers of species, the least apparent component is the biotic community of highest diversity—the soil microbiome. Numerous factors influence the composition and diversity of soil microbial communities, which in turn exert a profound impact on plant species occupying the soil and the biogeochemistry of essential plant nutrients. Of interest in forest ecosystems is how the soil microbiome interacts with the overstory, a phenomenon referred to as linkage. This study compared the soil microbiome of two adjacent stand types—hardwood- and longleaf pine (*Pinus palustris*)-dominated—and addressed the following questions: (1) How does soil microbiome vary with stand type? (2) Do the forest overstory community and soil microbiome exhibit linkage? Twelve 0.04-ha circular plots were established in each stand type to assess tree community composition and structure and to sample mineral soil for three separate analyses: assessment of soil fertility, measurement of total carbon and nitrogen (N), and extraction of genomic DNA for assessment of microbiome communities. All live stems ≥2.5 cm dbh in each plot were identified to species and measured for dbh to the nearest 0.1 cm. Mineral soil was taken from a depth of 5 cm and oven-dried at 38°C prior to analyses. Hardwood stands were dominated by flowering magnolia (*Magnolia grandiflora*) and southern evergreen oaks, whereas pine stands were dominated by longleaf pine and live oak (*Quercus virginiana*). Although soils of both stand types were highly acidic, the hardwood stands were generally higher in fertility, especially for total and available N. The overstory and soil microbial communities exhibited evidence of linkage among all sample plots combined. When assessed separately by stand type, only hardwood-dominated stands displayed evidence of overstory/microbial linkage. These results have broader implications for future scenarios given the sensitivity of soil microbes to climate change.

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KEYWORDS
hardwood forest, linkage, longleaf pine, soil microbiome

INTRODUCTION

Forest ecosystems comprise a paradox of biodiversity. Their most apparent component—the woody overstory—is actually the least diverse with respect to numbers of species, whereas the plant community of smallest physical stature—the herbaceous layer—can represent 80%–90% of forest plant diversity (Gilliam, 2007, 2014). Less apparent still is the biotic community of highest diversity—the forest microbiome (Mishra et al., 2020; Uroz et al., 2016), especially that of soil (Ji et al., 2022). Estimates have been made that 1 g of soil can support 10^{10}–10^{11} bacteria representing between 6000 and 50,000 species (van der Heijden et al., 2008).

Numerous abiotic and biotic factors determine the composition and diversity of soil microbial communities (Chen et al., 2022; Garbeva et al., 2004), which in turn exert a profound impact on plant species occupying the soil, including carbon (C) flux and the biogeochemistry of essential plant nutrients. This ecological feedback was termed a *circulus vitiosus* by Jenny et al. (1969). Fierer and Jackson (2006), on the other hand, used synoptic-scale data from throughout North and South America to conclude that what they called "microbial biogeography" was controlled predominantly by edaphic variables, especially soil pH, and that it operated in a way that was fundamentally different from the biogeography of "macro" species.

Spatially, soil microbiomes are highly nonuniform as a result of numerous biological, chemical, and physical parameters of the environment. Several recent studies have focused on the rhizosphere of dominant tree species of forest ecosystems, an important microbial hotspot (Ling et al., 2022). For example, Rosier et al. (2021) found that rhizosphere microbiomes were unique to individual tree species, especially in rural versus urban forests. Although they focused on woody species of bogs rather than forests, Boeraeve et al. (2022) found species-specific variation in bacterial composition of the rhizosphere. Using a meta-analysis of published studies, Liu et al. (2022) compared rhizosphere versus bulk soil and found higher microbial biomass and essential plant nutrients in the rhizosphere, especially in infertile soils. Ji et al. (2022) demonstrated the importance of soil fertility and plant species on soil bacterial communities in Korean pine forests. Thus, despite the contention of Fierer and Jackson (2006) that edaphic factors primarily affect soil microbiomes, it is clear that tree species can exert a profound influence on composition and biomass of soil microbial communities, especially at finer spatial scales (Prescott & Grayston, 2013).

Much of the recent work on the dynamics of soil microbiomes in forest ecosystems has been carried out on broad regional to global scales offering further insights, including responses of forest soil microbial communities to global climate change (Beugnon et al., 2021; Feng et al., 2022; Lladó et al., 2017). Whereas most of these have focused on bacterial and fungal microbes, others have examined soil protists (Fiore-Donno et al., 2022). Far less common are studies carried out at finer spatial scales, especially in adjacent forests of contrasting stand types, for example, hardwood- versus conifer-dominated forests. Among the few studies that have directly compared the soil microbiome of hardwood- versus pine-dominated stands, Frey et al. (2004) found that hardwood stands had approximately three times higher bacterial biomass than pine stands at Harvard Forest, USA.

Forest strata, especially the woody overstory and herbaceous layer, often interact in ways that lead to causal connections among them. For example, trees alter the light regime of forest herb communities (Neufeld & Young, 2014), whereas the herb layer can determine the survivorship of seedlings of overstory species (Elliott et al., 2015; George & Bazzaz, 2014). When this occurs, the strata are said to exhibit linkage. This has been reported for overstory and herbaceous strata for several forest ecosystem types (Gilliam, 2007; Jackson et al., 2012; McEwan & Muller, 2011; Vockenhuber et al., 2011). Linkage is commonly assessed using multivariate statistics, such as ordination. Axis scores from separate overstory and herb layer ordinations are subjected to correlation, with linkage evidenced by significant correlation between axis scores.

Although fewer linkage studies have included soil microbial communities in their analyses (Thoms et al., 2010; Thoms & Gleixner, 2013; Zhou et al., 2022), it is possible to employ similar multivariate analytical techniques as those used to assess overstory/herb layer relationships and apply them toward testing for linkage of forest vegetation with soil microbial communities. This can be particularly important with regard to processing of soil N, which not only affects plant communities but also involves a complex set of pathways involving soil microbes, especially bacteria (Frey et al., 2004). Working in a montane hardwood forest in West Virginia, USA, Gilliam et al. (2014) did not find evidence of a linkage among overstory and herbaceous strata, but found

linkage for forest vegetation and soil microbial communities. More specifically, they found that the nature of vegetation/microbe linkage varied with slope aspect. Linkage was detected between soil microbial and overstory communities only on the north slope. By contrast, it was evident between soil microbial and herb layer communities only on the south slope.

The University of West Florida (UWF) campus in Pensacola was initially constructed in 1963 from 405 ha of primarily second-growth longleaf pine (*Pinus palustris*) stands undergoing recovery from extensive logging in the Florida Panhandle (Knight et al., 2011). In his vision for design of the then-nascent campus, John E. Jarvis Jr. used a "design with nature" approach, emphasizing minimal cutting of trees during construction of buildings and establishment of roadways and parking lots (Jarvis, 2008). In addition, natural areas were established with an expansive network of numerous nonpaved, low-impact hiking trails.

Beginning in summer 2019, the unique nature of the UWF campus, including both the main campus and especially the associated natural areas, was recognized for its value in investigating the ecology of longleaf pine in the urban interface of a university campus (Cole & Bennington, 2017; Copenheaver et al., 2014; Roman et al., 2017; Turner, 1984). This led to the initiation of the UWF Campus Ecosystem Study (CES), which was conceived as an interconnected series of research investigations.

Most forest stands along the UWF campus nature trails are typical of second-growth longleaf pine forests experiencing chronic fire exclusion (Addington et al., 2015; Gilliam et al., 2021; Gilliam & Platt, 1999, 2006; Hiers et al., 2014; Provencher et al., 2003; Varner et al., 2005). These comprise stands of widely spaced longleaf stems primarily 50–100 years in age (Gilliam et al., 2022) with the otherwise fire-maintained open matrix (Noel et al., 1998; Platt, 1999; Platt et al., 1988) filled with hardwood species, especially numerous southern oaks, for example, live oak (*Quercus virginiana*), laurel oak (*Q. laurifolia*), water oak (*Q. nigra*), and blackjack oak (*Q. marilandica*).

Forest stands of a sub-catchment along sandy bluffs leading to Thompson Bayou were recently discovered to have no longleaf pine in the overstory. Reconnaissance revealed these stands to be dominated instead by mixed hardwood species (Figure 2). The close proximity of this sub-catchment with longleaf pine-dominated stands (hereafter, pine dominated) facilitates direct comparisons of the forest soil microbiome between contrasting stand types—hardwood versus pine—in adjacent areas, something generally lacking in the literature. Indeed, Lladó et al. (2017) reported a general lack of any soil microbial studies for the Panhandle region of Florida. In addition to filling this gap of knowledge for the region, further understanding of the relationship between soil microbiomes and forest overstories has broader implications for future scenarios with climate change, given (1) the central importance of soil microbes in the structure and function of forest ecosystems and (2) the sensitivity of soil microbes to changes in temperature (Jansson & Hofmockel, 2020).

The purpose of this study was to add two new dimensions to the ongoing UWF CES by (1) including stands dominated by hardwood species and (2) determination of the soil microbiome. The following questions were addressed: (1) How does soil microbiome vary with stand type? (2) Do the forest overstory community and soil microbiome exhibit linkage?

We predict the following outcomes with respect to these questions. We expect sharp differences in soil microbiome, with bacterial taxa associated in N processing (e.g., nitrification) being more prominent in soils of hardwood stands. Given the likelihood of greater spatial variation in tree species in hardwood-dominated stands, we expect to find overstory/soil microbe linkage for those stands, but not in pine-dominated stands.

MATERIALS AND METHODS

Study site

This study was carried out in forested stands off of trails within the Campus Side Trails area of UWF, Pensacola, Florida (30°33′8″ N, 87°13′ 29″ W) (Figure 1). Soils are predominantly of the Troup series. These consist of very deep, generally excessively drained soils that formed in sandy and loamy marine sediments (Hine, 2013). Soils of the Troup series are loamy, kaolinitic, thermic Grossarenic Kandiudults with a high seasonal water table below a depth of 2 m throughout the year (USDA, 2004). Previous work in forest stands adjacent to the study site has shown these soils to be acidic and infertile (Gilliam et al., 2021).

Field sampling

In each of the two stand types, 12 circular 0.04-ha plots were randomly located to assess forest stand composition and structure and to sample mineral soil (Figure 2). Within each plot, all living woody stems ≥2.5 cm dbh were identified to species and measured for dbh to the nearest 0.1 cm.

Mineral soil was taken to a 5-cm depth within each plot with a 2-cm diameter soil corer. Five cores were taken randomly from throughout the plot and combined in sterile polyethylene Whirl-Pak bags to yield three identical composite samples per plot to simultaneously integrate spatial heterogeneity within each plot and allow for

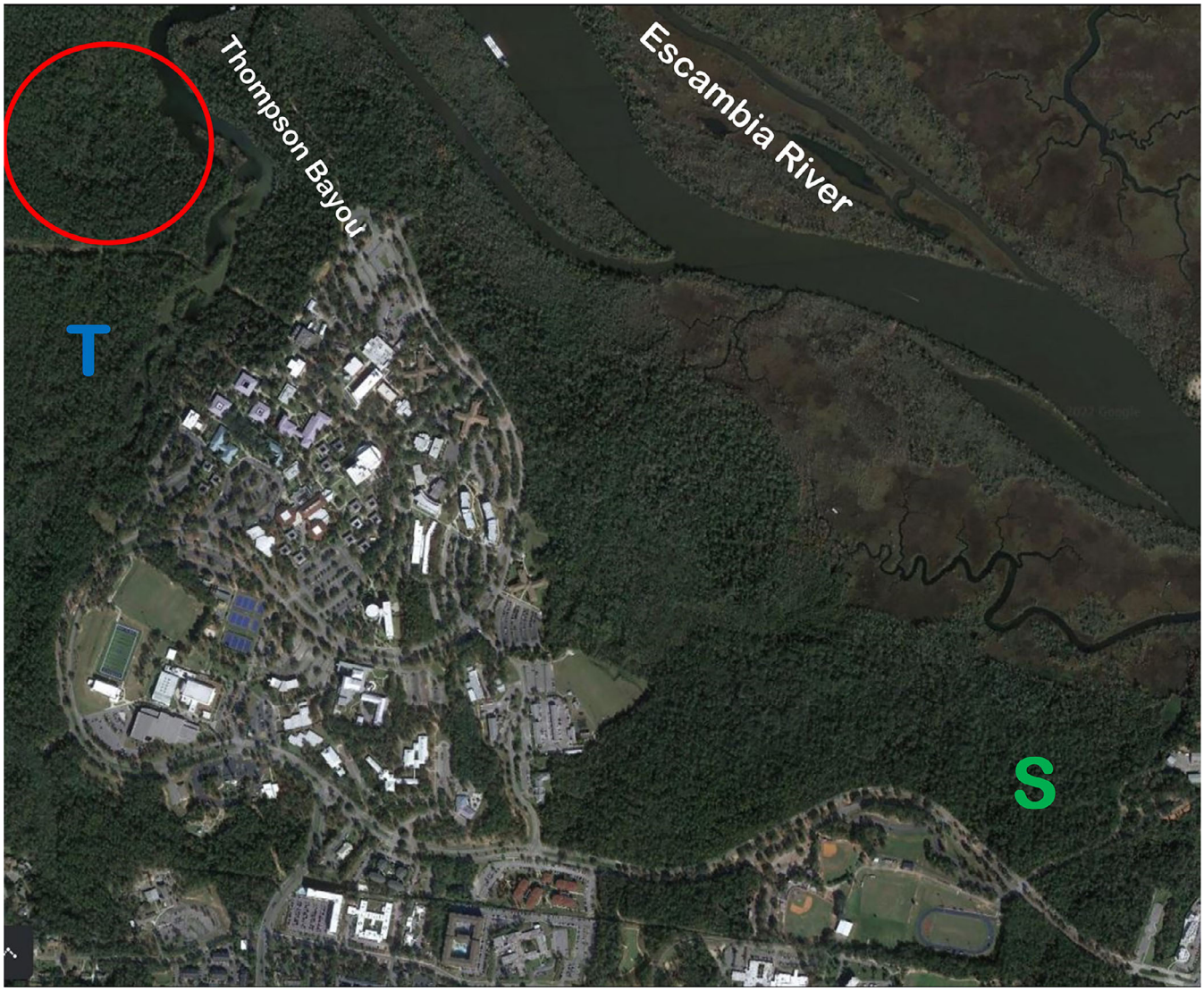

FIGURE 1 Aerial view of the campus of the University of West Florida, Pensacola, Florida (30.5102° N, 87.2125° W). The main campus comprises all roads, parking lots, and permanent structures, with university property extending to the wooded areas surrounding this. In the summer of 2019, all longleaf pines of the main campus ≥2.5 cm dbh were measured for dbh—a total of 2165 stems (Gilliam et al., 2021). In the summer of 2020, sample plots identical to those used in this study were established and sampled in two of the UWF campus natural areas: Edward Ball Trails (T) and Baars-Firestone Wildlife Sanctuary (S) (Gilliam et al., 2021). The area used for the present study is indicated in the red circle; see Figure 2 for specific plot locations.

three separate analyses, including (1) organic matter (OM), pH, and extractable nutrients, (2) total carbon (C)/N concentrations, and (3) extraction of genomic DNA for characterization of soil microbiome. All sampling equipment was sanitized between sample plots with a 70% ethanol solution.

Laboratory analyses

Immediately following sampling, soil was oven-dried to a constant weight at 38°C for quality-controlled, long-term storage. One bag from the three for each plot was shipped to the University of Maine Soil Testing Service and Analytical Laboratory. These samples were analyzed for OM (using loss on ignition at 375°C) and pH (distilled H_2O extraction). Following extraction with KCl, available NH_4^+ and NO_3^- were determined colorimetrically by flow injection analysis. Elemental analyses included P, Ca, Mg, and K determined via inductively coupled plasma optical emission spectrometry following extraction with ammonium acetate at pH 4.8 (modified Morgan extract—Jones, 2008).

A second bag from each plot was used for analysis of total soil C and N concentrations in the Soils Laboratory at the UWF Department of Earth and Environmental Science. Using standard procedures, each sample was

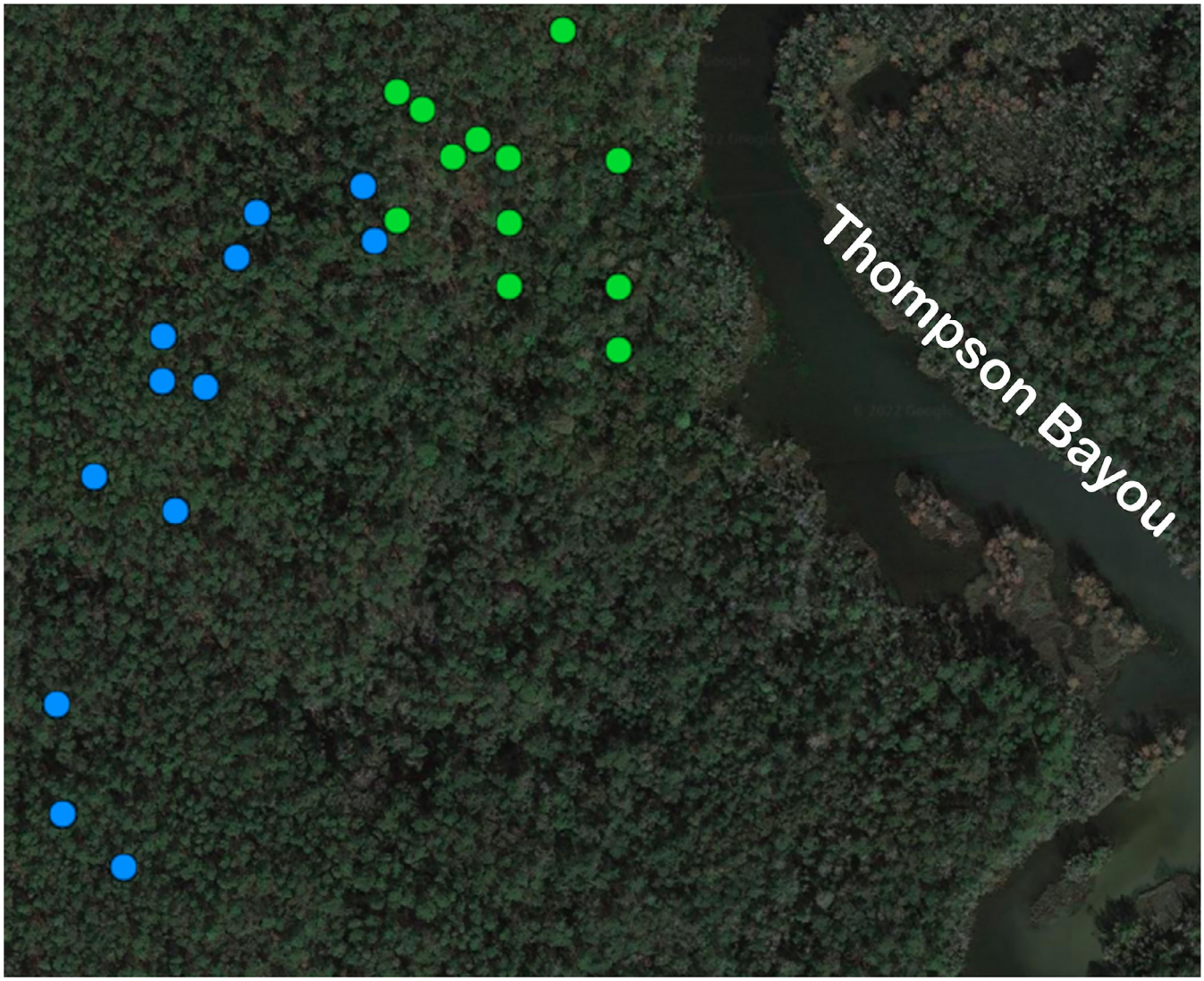

FIGURE 2 Map depicting locations of 400-m^2 circular plots for sampling of the hardwood-dominated (green) and longleaf pine-dominated (blue) stands.

measured for C and N with a ThermoFisher Scientific Flash 2000 N/C soil analyzer (Kopittke et al., 2017).

The third bag from each plot was used for assessment of the soil microbiome. DNA was extracted in duplicate for each plot using ~0.3 g of mineral soil with the QIAGEN DNeasy PowerSoil Kit according to instructions. DNA was semi-quantified using a ThermoFisher NanoDrop Spectrophotometer. Replicate samples ($n = 2$) were pooled and shipped to the University of Illinois at Chicago Sequencing Core for PCR amplification of the 16S rRNA gene using universal prokaryotic primers as previously described (Parada et al., 2016): 515F-Y (5′-GTG YCAGCMGCCGCGGTAA) and 926R (5′-CCGYCAATTY MTTTRAGTTT), and subsequent, paired-end deep sequencing using an Illumina MiSeq 739 platform.

16S rRNA gene sequences were processed via the analytical bioinformatic Quantitative Insights into Microbial Ecology (QIIME 2, v.2019.10) pipeline (Bolyen et al., 2019). The plugin "demux" was used to visualize interactive quality plots and inspect read quality. The plugin "DADA2" (Divisive Amplicon Denoising Algorithm, v.2; Callahan et al., 2017) was subsequently used to remove primers, truncate poor-quality bases, dereplicate reads, remove chimeras, and merge paired-end reads. The representative sequences of the amplicon sequence variants were taxonomically assigned with a Naïve Bayes classifier trained with the "feature-classifier" plugin at a 99% similarity to the SILVA database (v.138), as a reference (Quast et al., 2013).

Data analyses

To assess variation in forest communities among hardwood versus pine stand types, dbh data collected in the

field were converted to basal area for each measured stem. These were summarized by species and then combined with corresponding density to construct importance value tables for each stand type separately. Means of stand structural, diversity, and soil variables were compared between stand types via Student's *t* tests (Zar, 2009), with all statistical analyses performed with Statistix 9, Analytical Software, Tallahassee, FL. For a more thorough assessment of spatial variation in forest composition, soil microbiome, and soil fertility between stand types, tree species and microbiome taxa data were subjected to canonical correspondence analysis (CCA) with Canoco 5.11, Windows release (Šmilauer & Lepš, 2014) using soil data as environmental variables. Among the several ordination techniques, CCA has the advantage of providing a direct gradient analysis of environmental (i.e., soil) variables and species composition, in this case of both overstory and soil microbial communities (Barbour et al., 1999; Gilliam & Saunders, 2003).

Linkage between the forest overstory and soil microbial community was assessed following the methods described in Gilliam et al. (2014). To assess linkage across all sample plots in both stand types combined, CCA Axis 1 scores for the overstory ordination were compared with CCA Axis 1 scores for the soil microbiome ordination using Pearson product-moment correlation (Zar, 2009); this was repeated for CCA Axis 2 scores. To determine whether linkage varied with stand type, additional overstory and microbiome CCA ordinations were performed for each stand type separately. Again, linkage was determined by testing for relationships among CCA axis scores for overstory and microbial communities using Pearson product-moment correlation.

The field and statistical design of this study is an example of simple pseudoreplication, a common challenge among field studies comprising large areas, with each stand type representing a sample size of one (Hurlbert, 1984); thus, our data should be interpreted with that in mind. It is our contention that any differences we report in soil variables are stand-driven, rather than preexisting differences among sites, given that these soils are of the same series (Troup series) derived from the same parent material (Hine, 2013).

RESULTS AND DISCUSSION

Stand composition and soil fertility

Unsurprisingly, given the criteria for plot location, hardwood species dominated the hardwood stands and longleaf pine dominated the pine stands. Similar to previous studies at this site (e.g., Gilliam et al., 2021), live oak was the ubiquitous species, being second in importance in both stand types, whereas flowering magnolia (*Magnolia grandiflora*) and longleaf pine were the dominant species in hardwood- and pine-dominated stands, respectively (Tables 1 and 2). Indeed, the pine stand exhibits characteristics of chronically unburned longleaf pine ecosystems (Kirkman & Jack, 2018), with >80% of stems being hardwood species occupying the open spaces otherwise maintained by fire. Chronic fire exclusion has numerous other effects on longleaf ecosystems not measured in this study, including forest floor characteristics and ground cover plants (Cipollini et al., 2012, 2019; Hiers et al., 2007; Platt et al., 2006).

Soil fertility varied between stand types. Although pH was not significantly different between sites, OM, total C and N, and extractable N (both NO_3^- and NH_4^+), K, Mg, and P were significantly higher in hardwood-dominated soils (Table 3). Differences in soil fertility in conifer versus hardwood are well documented (Heděnec et al., 2023; Schroth et al., 2007) and arise from several factors related to differences in foliar nutrients, most of which are higher in hardwood foliar biomass (Marschner, 2012). Although foliar N is not always lower in conifers, lignin—an important control on N mineralization—is typically higher, thus resulting in lower available N (Heděnec et al., 2023).

As described previously (see *Study site*), soils of both stand types are of the Troup series, which are characteristically deep, excessively drained, and acidic sands (Hine, 2013). As a result, fertility is primarily driven by OM, which was ~70% higher in hardwood soils, but low for both stand types. For example, these values were 2.2% and 3.8% for pine- and hardwood-dominated stands (Table 3), whereas OM in soils of mesic eastern hardwood sites is typically 10%–12% (Gilliam et al., 2014, 2018). This is also consistent with contrasts in general fertility wherein small variation in OM can bring about disproportional variation in extractable nutrients, such as N, P, and base cations. Although lower than in soils of hardwood stands, values of virtually all measures of fertility of soils from the pine-dominated stands were significantly higher than those of other pine-dominated sites of the UWF natural areas studied previously (see Gilliam et al., 2021 for data). Again, it is likely that OM for the pine-dominated soil (2.2%) is higher than in the other sites (1.4%) (Gilliam et al., 2021).

CCA revealed patterns of similarities and contrasts regarding species variation and responses of overstory composition to environmental gradients. Sample plots were separated widely in ordination space with respect to stand type along Axis 1, with greater variability among hardwood stand plots (i.e., greater scatter in ordination space) than among pine plots (Figure 3). Consistent with

TABLE 1 Importance values (IVs) for woody species in the hardwood stand.

Species	BA (m^2/ha)	*D* (stems/ha)	Relative BA (%)	Relative *D* (%)	IVs (%)
Magnolia grandiflora	3.34	327	17.1	21.2	19.2
Quercus virginiana	4.34	185	22.3	12.0	17.1
Q. laurifolia	2.79	215	14.3	13.9	14.1
Ostrya virginiana	1.41	211	7.2	13.7	10.4
Q. marilandica	2.01	17	10.3	1.1	5.7
Vaccinium arboreum	0.39	135	2.0	8.8	5.4
Fagus grandifolia	1.32	29	6.8	1.9	4.3
Carya glabra	1.15	26	5.9	1.7	3.8
Hamamelis virginiana	0.10	100	0.5	6.5	3.5
Halesia carolina	0.77	44	3.9	2.8	3.4
Ilex vomitoria	0.31	77	1.6	5.0	3.3
Q. nigra	0.40	44	2.1	2.8	2.4
M. virginiana	0.13	54	0.7	3.5	2.1
Pinus rigida	0.44	29	2.2	1.9	2.1
P. serotina	0.18	10	0.9	0.7	0.8
Oxydendrum arboreum	0.26	2	1.3	0.1	0.7
Prunus caroliniana	0.07	10	0.3	0.7	0.5
I. opaca	0.01	15	0.1	0.9	0.5
Q. alba	0.04	6	0.2	0.4	0.3
Nyssa sylvatica	0.02	2	0.1	0.1	0.1
Morus rubra	0.01	2	0.0	0.1	0.1
Total	19.5	1546	100.0	100.0	100.0

Note: IVs of each species are based on the mean of relative basal area (BA) and relative density (*D*).

contrasts previously discussed regarding OM, and based on vector length, environmental variables important in explaining species variation between stand types were associated with soil OM, including base cation availability (especially K^+), extractable NO_3^-, and extractable P. Variation among plots within stand types was primarily driven by soil pH and extractable Al^{3+} (Figure 3).

Among overstory species, longleaf pine and sweetgum (*Liquidambar styraciflua*) were the most distant from the center of the CCA axes and were the only species unique to the pine-dominated stands (Figure 3). At the center of these axes are species of high relative importance in both stands, including live oak, farkleberry (*Vaccinium arboretum*), and flowering magnolia (*M. grandiflora*) (Figure 3), the latter of which was not only dominant in hardwood stands, but also was third in importance in the pine stands (Tables 1 and 2). Numerous species were unique to hardwood stands, including American beech (*Fagus grandifolia*), mulberry (*Morus rubra*), sourwood (*Oxydendrum arboretum*), pond pine (*Pinus serotina*), pitch pine (*P. rigida*), and Carolina cherry (*Prunus caroliniana*).

Soil microbiome

Three microbial taxa varied significantly between stand types—*Deltaproteobacteria*, *Thermogemmatisporaceae*, and *Syntrophobacteraceae*—the former higher in hardwood stands and the latter two higher in pine stands (Table 4). Other than these, and contrary to our expectations, soil microbial communities did not display sharp contrasts in composition between hardwood- versus pine-dominated stands. Among the more dominant taxa in the soil of both stand types were *Alphaproteobacteria*, *Rhodospirillaceae*, *Isosphaeraceae*, *Mycobacterium*, *Bradyrhizobiaceae*, *Actinomycetales*, and *Gemmataceae*.

In a comprehensive review, Lladó et al. (2017) identified bacteria common to forest soils that included the taxa found in our study. They ascribed the widespread nature of these bacterial groups in soil to both their functional importance to forest ecosystems, as well as their sensitive response to environmental gradients, especially soil pH. Although our sampling did not discern between bulk soil and the rhizosphere of tree roots, several of the important taxa in our study are known to be closely associated with

TABLE 2 Importance values (IVs) for woody species the pine stand.

Species	BA (m^2/ha)	*D* (stems/ha)	Relative BA (%)	Relative *D* (%)	IVs (%)
Pinus palustris	12.74	154	49.7	18.5	34.1
Quercus virginiana	7.71	267	30.1	32.0	31.0
Magnolia grandiflora	1.92	121	7.5	14.5	11.0
Vaccinium arboreum	0.46	108	1.8	13.0	7.4
Q. nigra	0.91	60	3.5	7.2	5.4
Q. laurifolia	1.00	19	3.9	2.3	3.1
Ilex vomitoria	0.03	35	0.1	4.3	2.2
Q. marilandica	0.34	8	1.3	1.0	1.2
Carya glabra	0.21	10	0.8	1.3	1.0
Ilex opaca	0.02	13	0.1	1.5	0.8
Ostrya virginiana	0.19	6	0.8	0.7	0.8
Hamamelis virginiana	0.02	8	0.1	1.0	0.5
Halesia carolina	0.01	8	0.0	1.0	0.5
Magnolia virginiana	0.01	6	0.0	0.7	0.4
Nyssa sylvatica	0.01	4	0.0	0.5	0.3
Liquidambar styraciflua	0.06	2	0.2	0.3	0.2
Q. alba	0.00	2	0.0	0.3	0.1
Total	25.6	833	100.0	100.0	100.0

Note: IVs of each species are based on the mean of relative basal area (BA) and relative density (*D*).

TABLE 3 *T* test comparisons for means of soil variables between hardwood- and longleaf pine-dominated (pine) stands.

Site	pH	OM (%)	C (%)	N (%)	NO_3-N (mg/kg)	NH_4-N (mg/kg)	Ca (mg/kg)	K (mg/kg)	Mg (mg/kg)	P (mg/kg)
Hardwood	4.63 ± 0.09	3.78 ± 0.38	2.34 ± 0.27	0.11 ± 0.01	0.32 ± 0.02	8.19 ± 0.98	74.1 ± 17.7	21.4 ± 2.1	15.3 ± 2.5	0.99 ± 0.12
	NS	**	**	**	***	*	NS	***	*	**
Pine	4.55 ± 0.07	2.23 ± 0.19	1.37 ± 0.14	0.07 ± 0.01	0.20 ± 0.01	5.28 ± 0.56	35.9 ± 10.5	11.6 ± 1.0	8.9 ± 1.0	0.53 ± 0.04

Note: NS indicates means not significantly different at $p < 0.05$.
Abbreviation: OM, organic matter.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

rhizospheres, for example, *Alphaproteobacteria* and *Actinomycetales* (Hugoni et al., 2021).

Among taxa that varied significantly between stand types, members of the *Deltaproteobacteria* are widely prominent in forest soils, but have also been shown to be responsive to environmental gradients, including slope aspect and N availability (Gilliam et al., 2011, 2014). Our results are consistent with these findings, as *Deltaproteobacteria* were higher in soils with higher total and available N (Table 3). Wu et al. (2017) found members of *Thermogemmatisporaceae* to be common in mineral soil and to increase in prominence with higher elevation and lower pH. These observations generally support our results, especially regarding elevation, as mean elevation was significantly higher ($p < 0.0001$) for pine stands than for hardwood stands (20.0 ± 0.5 vs. 10.4 ± 1.4 m, respectively). Finally, Hudz and Skivka (2021) found members of the *Syntrophobacteraceae* to be highly sensitive to soil fertility, as they appear to be for our sites.

Linkage between overstory and soil microbiome

In addition to these patterns of similarities and differences in soil microbial communities in hardwood- and pine-dominated forest soils, the principal focus of this study was to determine whether these communities exhibit linkage with overstory communities, something assessed via correlation of axis scores of CCA ordination (see *Materials and methods*). Ordination analysis reveals patterns of species variation such that significant

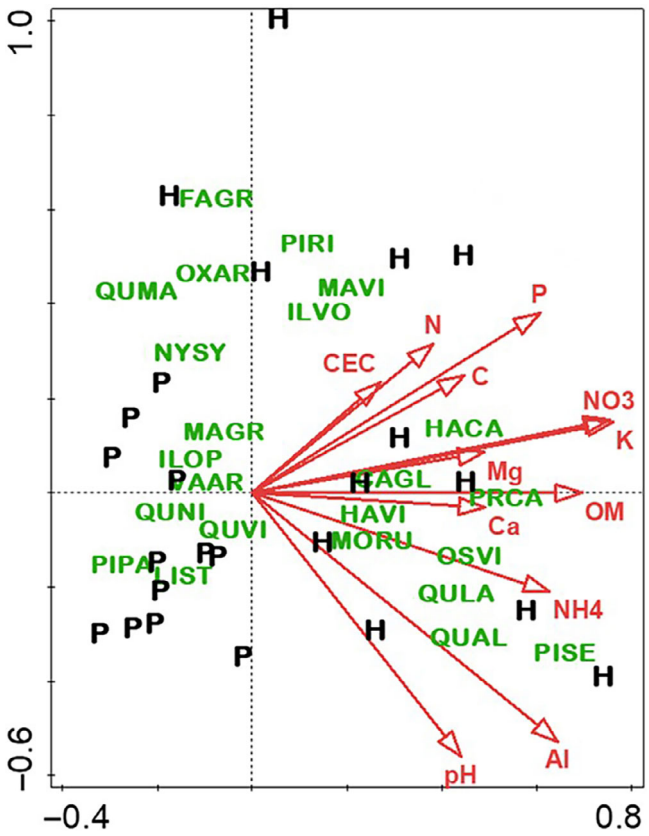

FIGURE 3 Canonical correspondence analysis of overstory species in hardwood (H) and longleaf pine-dominated stands (P). For vectors, element symbols are extractable concentrations of stated elements, "CEC" is cation exchange capacity, "OM" is organic matter, "pH" is H_2O-extractable soil pH, and "C" and "N" are total C and N, respectively. Species are indicated by four-letter codes: *Carya glabra* (CAGL), *Fagus grandifolia* (FAGR), *Halesia carolina* (HACA), *Hamamelis virginiana* (HAVI), *Ilex opaca* (ILOP), *Ilex vomitoria* (ILVO), *Liquidambar styraciflua* (LIST), *Magnolia grandifolia* (MAGR), *Magnolia virginiana* (MAVI), *Morus rubra* (MORU), *Nyssa sylvatica* (NYSY), *Ostrya virginiana* (OSVI), *Oxydendrum arboreum* (OXAR), *Pinus palustris* (PIPA), *Pinus rigida* (PIRI), *Pinus serotina* (PISE), *Prunus caroliniana* (PRSE), *Quercus alba* (QUAL), *Quercus falcata* (QUFA), *Quercus laurifolia* (QULA), *Quercus marilandica* (QUMA), *Quercus nigra* (QUNI), *Quercus virginiana* (QUVI), and *Vaccinium arboretum* (VAAR).

correlations among ordination axis scores of forest strata provide evidence of linkage between strata. As discussed in Gilliam (2007), linkage should arise when forest communities respond to similar gradients in a similar fashion, something that has been reported in the literature for overstory and herbaceous layer communities (McEwan & Muller, 2011; Vockenhuber et al., 2011). Because CCA is a type of direct gradient analysis (Barbour et al., 1999; Šmilauer & Lepš, 2014), when linkage is evident (i.e., a significant correlation), examination of CCA ordination space can also reveal environmental gradients that may have led to linkage.

TABLE 4 Top 25 microbial taxa for soils from hardwood- versus pine-dominated stands.

Taxon	Hardwood	Pine
Alphaproteobacteria	1889 ± 285	1759 ± 245
Rhodospirillaceae	1741 ± 273	1896 ± 363
Isosphaeraceae	1703 ± 330	1415 ± 201
Mycobacterium	1576 ± 250	1488 ± 132
Bradyrhizobiaceae	1466 ± 226	1340 ± 190
Actinomycetales	1373 ± 227	1299 ± 186
Gemmataceae	1071 ± 207	826 ± 231
Gaiellales	906 ± 135	859 ± 115
Sinobacteraceae	822 ± 171	801 ± 141
Koribactereaceae	716 ± 109	684 ± 126
Bacillus	554 ± 182	651 ± 558
Ellin6513	533 ± 101	533 ± 92
Acidobacteriaceae	429 ± 93	413 ± 60
Myxococcales	408 ± 66	448 ± 112
Chthoniobacteraceae	403 ± 91	538 ± 141
Betaproteobacteria	345 ± 95	394 ± 120
Deltaproteobacteria	336 ± 83*	238 ± 33
Thermogemmatisporaceae	289 ± 72*	392 ± 87
DA101	248 ± 47	249 ± 62
WPS2	227 ± 56	238 ± 50
Planctomyces	187 ± 36	179 ± 44
JG30KFAS9	160 ± 36	173 ± 43
Paenibacillaceae	130 ± 23	170 ± 120
Rhizobiales	116 ± 29	144 ± 30
Syntrophobacteraceae	97 ± 22*	136 ± 30

Note: Values shown are means ± 1 SE of amplicon sequence variants for each taxon.
*Means significantly different between stand types at $p < 0.05$.

Although the correlation between Axis 1 of the overstory ordination (Figure 2) and that of the soil microbiome (Figure 3) was not significant, it was significant between Axis 2 of overstory and that of soil microbiome ($p < 0.05$) (Table 5A). Thus, we conclude that the overstory and soil microbial communities are linked. This supports the conclusions of several studies that have shown overstory/microbial linkage (Gilliam et al., 2014; Heděnec et al., 2023; Thoms & Gleixner, 2013; Zhou et al., 2022). Further examination suggests that linkage arose from responses to gradients in soil pH (Table 5A).

When run for each stand type separately, the results of CCA (graphical data not shown) further suggest that linkage for our site is stand-specific, being evident only in hardwood-dominated stands. Indeed, this supports our initial expectation. Neither Axis 1 nor Axis 2 for overstory

TABLE 5 Correlation matrix for canonical correspondence analysis (CCA) axis scores as indicated and soil pH: (A) for all plots combined and (B) for hardwood-dominated stands only.

CCA axis/pH	Tree1	Tree2	Microbe1	Microbe2
(A) All plots				
Tree2	−0.0100			
	0.9629			
Microbe1	0.0285	−0.0993		
	0.8947	0.6442		
Microbe2	0.2499	**−0.4834**	0.0561	
	0.2388	**0.0167**	0.7945	
pH	**0.4508**	**−0.4574**	0.1124	**0.8229**
	0.0271	**0.0246**	0.6011	**0.000**
(B) Hardwood				
Tree2	0.1112			
	0.7308			
Microbe1	**−0.6674**	0.2211		
	0.0177	0.4898		
Microbe2	0.3361	0.2605	−0.0040	
	0.2854	0.4134	0.9902	
pH	**−0.8059**	−0.537	**0.8989**	−0.0572
	0.0015	0.8683	**0.0001**	0.8598

Note: The top value in each pair is Pearson product-moment correlation coefficient (r) and the corresponding p value appears below. Significant correlations at $p < 0.05$ are indicated by boldface.

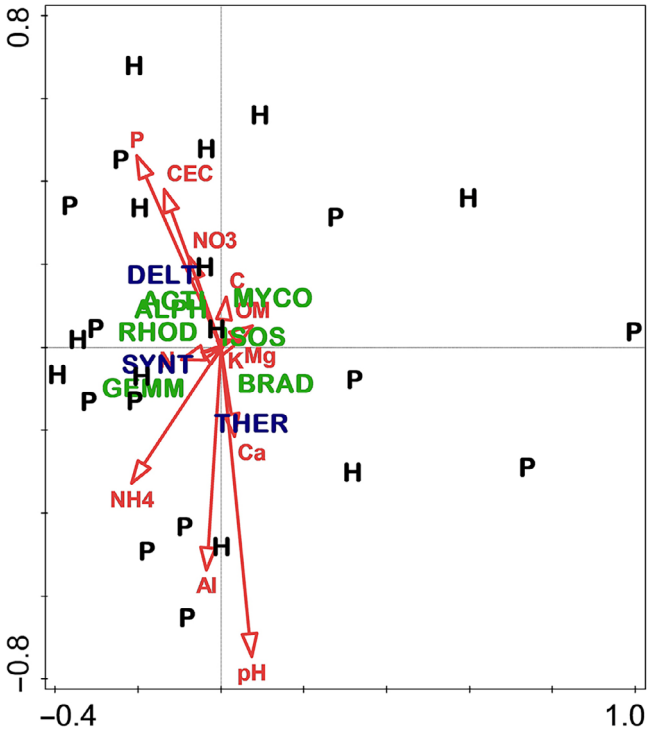

FIGURE 4 Canonical correspondence analysis of soil microbiome in hardwood (H) and longleaf pine-dominated stands (P). For vectors, element symbols are extractable concentrations of stated elements, "CEC" is cation exchange capacity, "OM" is organic matter, "pH" is H_2O-extractable soil pH, and "C" and "N" are total C and N, respectively. Taxa are indicated by four-letter codes: *Alphaproteobacteria* (ALPH), *Rhodospirillaceae* (RHOD), *Isosphaeraceae* (ISOS), *Mycobacterium* (MYCO), *Bradyrhizobiaceae* (BRAD), *Actinomycetales* (ACTI), *Gemmataceae* (GEMM), *Deltaproteobacteria* (DELT), *Thermogemmatisporaceae* (THER), and *Syntrophobacteraceae* (SYNT). Violet font indicates taxa significantly different between stand types (see Table 5).

versus soil microbiome for the pine-dominated stand yielded significant correlations (data not shown). By contrast, Axis 1 scores for overstory versus soil microbiome for the hardwood-dominated stand were significant ($p < 0.05$) (Table 5B). As with the analysis for all plots combined, linkage for the hardwood stand appears to have arisen from responses to a soil pH gradient (Table 5B).

Synthesis and conclusions

A broader implication for the research summarized herein—as part of the ongoing UWF CES—is the heuristic value of college/university campuses as sites for ecological study. Campuses represent a unique urban interface with their spatial array of permanent structures, paved surfaces, and green spaces (Copenheaver et al., 2014; Roman et al., 2017; Turner, 1984). For longleaf pine in particular, Gilliam et al. (2021) identified several institutions within the species' natural range, including Berry College (Georgia), Valdosta State University (Georgia), Stetson University (Florida), Florida A&M University, Florida State University, University of Central Florida, and Rollins College (Florida), with appreciable coverage of longleaf pine and where research/restoration efforts are planned or are underway (Cipollini et al., 2012, 2019; Cole & Bennington, 2017). The results of our study reveal further insights into the ecology of longleaf pine in the context of an urban interface, particularly with respect to longleaf ecosystems experiencing chronic fire exclusion (Varner et al., 2005).

Although the general similarity of the soil microbiome between stand types did not support our expectations, we found evidence of linkage between the forest overstory community and the soil microbial community. We further expected that this would also vary between stand types, with linkage occurring for hardwood-, but not pine-dominated, stands, an outcome supported by our findings.

It is notable that soil pH appeared to be a principal factor underlying patterns of overstory/microbial linkage,

considering that we found neither a significant difference in pH between stand types (Table 3) nor a separation of plots in ordination space along Axis 1 for both overstory (Figure 3) and soil microbiome (Figure 4). Instead, the spread of plots for both ordinations was along the vector for pH, which was more closely aligned with Axis 2. It is further possible that the significant correlations of Axis 2 of overstory with those of soil microbiome (Table 5A), as evidence of general linkage, were driven primarily by correlations of axis scores for the hardwood-dominated plots, which were both highly correlated with soil pH (Table 5B).

Certainly, enumerable factors influence the composition and diversity of both soil microbial communities and forest overstory communities that they support (Chen et al., 2022; Garbeva et al., 2004). The multiple dynamic feedbacks between these two integral components of forest ecosystems represent what Jenny et al. (1969) famously called a *circulus vitiosus*, wherein it is often difficult to determine which component has a more versus less profound influence on the other. Regardless, our results underscore the importance of mineral soil in maintaining the structure and function of forest ecosystems.

AUTHOR CONTRIBUTIONS

Frank S. Gilliam conceived the research. Elizabeth A. Hargis, Sarah K. Rabinowitz, Brenton C. Davis, Leo L. Sweet, and Frank S. Gilliam collected the field data and soil samples. Elizabeth A. Hargis, Sarah K. Rabinowitz, Brenton C. Davis, Leo L. Sweet, and Joseph A. Moss performed gDNA extractions. Joseph A. Moss performed the bioinformatics. Frank S. Gilliam analyzed the data. Frank S. Gilliam, Elizabeth A. Hargis, Sarah K. Rabinowitz, Brenton C. Davis, Leo L. Sweet, and Joseph A. Moss wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Gilliam, 2022) are available from the University of West Florida Argo Scholar Commons repository: https://ircommons.uwf.edu/esploro/outputs/99380127797206600.

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