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Soil microorganisms respond distinctively to adaptive multi-paddock and conventional grazing in the southeastern United States

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Abstract

Variable results from studies of the associations between grazing management and soil microbiological properties in grasslands suggest the need for further investigation. We assessed the response of soil microbiological properties to conventional and adaptive multi-paddock (AMP) grazing management practices at paired farms at five locations in the southeastern United States. We measured total DNA and abundances of fungi and bacteria by quantitative polymerase chain reaction (qPCR) as proxies for soil microbial biomass, potential carbon, nitrogen, and phosphorus cycling activities using qPCR of functional genes, and carbon mineralization activities by respiratory assays. Abundances of fungi ($p = 0.009$) and bacteria ($p = 0.001$) were greater under AMP management compared to conventional management; however, there was no difference in the fungal/bacterial ratios between management practices. Gene copies encoding for nitrification, denitrification, and phosphate hydrolysis were significantly greater ($p < 0.05$) under AMP compared to conventional management. Basal soil respiration was elevated ($p = 0.004$) under AMP compared to conventional management presumptively due to the greater abundance of microbes that were actively transforming plant exudates and residues. In contrast, labile carbon limitation of respiratory activities was greater ($p \leq 0.01$) under conventional compared to AMP management indicating decreased processing of soil carbon and formation of microbial biomass. Using discriminant function analysis, the 19 biological response variables successfully classified 94% of the 180 soil samples according to grazing management. In summary, AMP grazing management influenced the numbers and key activities of soil microbes in a distinctive manner that is associated with the retention of soil organic carbon and nitrogen reported in these grazed grasslands.

1 | INTRODUCTION

Soil microorganisms are an inherent component of healthy grassland soils capable of supporting forage production, livestock production, and ecosystem services and they respond

Abbreviations: AMP, adaptive multi-paddock grazing; CG, conventional grazing; qPCR, quantitative polymerase chain reaction; SIR, substrate-induced respiration; SOC, soil organic carbon.

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to grazing management (Eldridge et al., 2017; Macdonald et al., 2015). Knowledge of soil microbial properties promotes understanding of mechanisms for management-induced changes in soil physicochemical properties, such as soil organic carbon (SOC) concentrations, and will contribute to modeling relationships between soil conditions and long-term forage/animal production and delivery of ecosystem services. There is a need to identify dynamic soil indicators that are responsive to grazing management and relate to the functions of grazing land soils to improve the value of grazing land soil health assessment practices (Brown & Herrick, 2016).

Research results on long-term livestock grazing management effects on soil microbes vary with edaphoclimatic conditions, experimental design, grazing management practices and their duration, and the analyses conducted. Heavy continuous grazing has been reported to reduce soil microbial biomass and activities (Bardgett et al., 2001; Ingram et al., 2008; Zhao et al., 2017). In some cases, livestock grazing has increased soil microbial biomass, microbial activities, and altered microbial community structure (Bardgett et al., 1997, 2001; Pan et al., 2018; Patra et al., 2005; Wang & Tang, 2019), including under rotational grazing management that incorporates a rest period for the grassland (Kleppel, 2019; Teague et al., 2011). Grazing has been reported to increase forage primary production by overcompensation responses to herbivory including increased root exudation that stimulates soil microbial activities and increases plant-available nutrients (Hamilton III & Frank, 2001). Increased forage production, root exudation, intermittent soil and plant disturbance by livestock, and distribution of urine and manure should increase soil microbial biomass and promote carbon (C) and nutrient cycling that would explain increased SOC and nutrient retention reported under rotational grazing management (Byrnes et al., 2018).

Adaptive multi-paddock (AMP) grazing is a form of rotational grazing that utilizes a large number of small paddocks, high stocking densities, short grazing periods (e.g., 1–4 days), and long resting periods for forage regrowth (Mosier et al., 2021; Teague et al., 2013). Paddocks are not permanent, and grazing and resting periods are adjusted according to forage condition. In contrast, conventional grazing (CG) is defined for the purpose of this study as continuous grazing at low or moderate stocking densities with infrequent movement of cattle among established paddocks. As part of a multidisciplinary, field-scale research project studying grasslands in the southeastern United States grazed under long-term AMP or CG management (Mosier et al., 2021), we collected soil samples from the same sampling transects on these farms for soil microbial analyses. Recently published work on these same farms reported that AMP grazing management resulted in greater soil C and nitrogen (N) stocks (Mosier et al., 2021), soil fertility (Mosier et al., 2022), and standing crop (forage) biomass (Apfelbaum et al., 2022) compared to CG management. Our objectives were to (1) determine if these long-term grazing management practices differentially influenced soil microbial biomass and cycling of C, N, and phosphorus (P), (2) evaluate selected microbial taxa as potential indicators of grazing management, (3) determine by discriminant function analysis if the set of soil biological indicators were effective at discriminating grazing management practices, and (4) determine the relative effectiveness of each soil biological indicator for discriminating management practices. These data address knowledge gaps regarding the response of soil microorganisms to grazing management, their potential as biological indicators of rangeland health, and their role in mechanistic hypotheses for C and nutrient dynamics in managed grasslands. Based on increased SOC reported under rotational grazing management under some climatic conditions (Byrnes et al., 2018), we hypothesized that soil microbial biomass and activities would be greater under AMP grazing management compared to conventional management.

Core Ideas

- Adaptive multi-paddock grazing increased soil bacterial and fungal populations relative to conventional grazing.
- Adaptive multi-paddock grazing increased indicators of carbon (C) and nutrient cycling relative to conventional grazing.
- Discriminant analysis using 19 soil biological variables classified 168 of 179 soil samples by grazing management.
- Soil microbial response to grazing management was associated with soil C and N retention reported at these sites.

2 | MATERIALS AND METHODS

2.1 | Study sites

The response of soil microbiological properties to long-term grazing management was measured at five locations arranged in a north–south transect from southern Kentucky to southern Mississippi (Table 1).

At each location there were two farms paired by grazing management, AMP and CG, in close proximity to each other. At three of the five locations, farm pairs were immediate neighbors. Detailed information on the farm selection process, including matching soil types and slopes, is described in Apfelbaum et al. (2022). For this study, AMP management had >40 paddocks, stocking densities >60 animal units

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TABLE 1 Farm pair locations, mean annual temperature (MAT), and precipitation (MAP).

Farm pair	Farm location	MAT (°C)	MAP (mm)	Grazing practice	Slope (%)	Soil series	Soil taxonomy	A-horizon depth (cm)[a]	A-horizon texture, % sand, silt, clay
1	Adolphus, KY	13.8	1316	AMP	2–6	Trimble gravelly silt loam	Fine-loamy, siliceous, semiactive, Mesic Paleudults	13.9 (0.6)	16.7, 52.6, 30.7
					6–12				
				CG	2–6			13.5 (0.4)	25.9, 49.6, 24.5
					6–12				
2	Sequatchie, TN	14.2	1432	AMP	0–2	Emory silt loam	Fine-loamy, siliceous, semiactive, Typic Paleudults	14.4 (0.8)	30.4, 35.9, 33.8
					2–5	Cumberland silty clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudults		
				CG	0–2	Emory silt loam	Fine-loamy, siliceous, semiactive, Typic Paleudults	11.9 (0.5)	43.1, 28.3, 28.6
					2–5	Cumberland silty clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudults		
3	Fort Payne, AL	15.1	1420	AMP	2–6	Hartsell fine sandy loam	Fine-loamy, siliceous, semiactive, Typic Hapludults	13.3 (0.4)	64.4, 21.0, 14.7
					6–10				
				CG	2–6	12.1 (0.4)	70.0, 15.0, 15.0		
					6–10				
4	Piedmont, AL	15.7	1352	AMP	2–6	Cumberland gravelly loam	Fine, kaolinitic, thermic, Rhodic Paleudults	11.7 (0.5)	47.3, 26.0, 26.7
					6–10	Cumberland gravelly clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudults		
				CG	2–6	Cumberland gravelly loam	Fine, kaolinitic, thermic, Rhodic Paleudults	12.0 (0.4)	54.8, 24.6, 20.6
					6–10	Cumberland gravelly clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudults		
5	Woodville, MS	19.0	1649	AMP	2–5	Loring silt loam	Fine-silty, mixed, active, thermic Oxyaquic Fagiudalfs	9.9 (0.5)	23.8, 56.6, 19.6
					5–8				
				CG	2–5	9.2 (0.4)	18.4, 64.3, 17.3		
					5–8				

Note: Slope and soil characteristics for sampling locations at each farm separated by grazing practice, adaptive-multipaddock grazing (AMP), and conventional grazing (CG) management. Reprinted with permission from Mosier et al., 2021.

[a]Average ± (standard error).

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ha^{-1}, stocking rates >1 animal unit ha^{-1}, and a rest/grazed ratio >40 days for the grasslands. The CG farms had values less than these management thresholds with the exception of one location which had a stocking rate of 1.09 animal unit ha^{-1}. Animal unit was defined as an adult, 450 kg beef cow and the rest:grazed ratio was the number of days an area of grassland was grazed annually divided by the number of days the same area was rested annually. Detailed information on grazing management including paddock size, stocking density, and so forth, for each farm is provided in Table S1 and further description of these study sites is available in Mosier et al. (2021). Detailed information on the vegetation present at each location is provided in Table S2 and further description of the influence of grazing management on grassland vegetation is available in Apfelbaum et al. (2022).

2.2 | Soil sampling

At each farm, sampling transects (average 75-m wide) were established at three landscape zones (upper, middle, and lower) within each of two representative catenas. One catena consisted of relatively flat ground and the other was sloping ground at each farm; see Table 1 for slopes. The selection of catenas and establishment of the sampling transects were conducted to match soil type and slope for each farm pair (Table 1). Soil samples were collected at both ends and the middle of each of the six transects. At each of the 18 sampling points, six individual 3.1-cm diameter hand cores (0–15 cm depth) were collected, composited, and thoroughly mixed. In the field, each of the 18 composite soil samples collected per farm were split into two sealed bags, one placed immediately on ice packs for activity assays and the other on dry ice for nucleic acid analyses. Storage conditions were maintained during transportation until reaching the laboratory where samples were stored at 4°C or −80°C, depending on analysis. Soil sampling was conducted May 2018 to June 2018. A total of 180 soil samples from the 10 farms were subjected to microbiological analyses.

2.3 | DNA extraction and quantification

Soil samples were removed from storage (−80°C) on the day of processing, thawed, sieved (4.75 mm) at field moisture, visible roots removed, and thoroughly mixed. Soil moisture was measured gravimetrically on a dry-weight basis (24 h, 105°C) in triplicate for each sample. For each well-mixed sample, DNA was extracted and purified from 10 g soil using the DNeasy PowerMax Soil Kit (Qiagen Inc.) following the manufacturer's protocol. Extracted DNA was quantified using the Invitrogen Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) following the manufacturer's protocol using triplicate standard curves for each batch. Sample DNA was assayed in triplicate on three extract dilutions per sample to assure the correct working range for each extract. Fluorescence readings were acquired using a Biotek FLx800TBI microplate reader (excitation: 485/20, emission: 528/20). Total DNA (ng g^{-1} dry soil) was used as a proxy for microbial biomass (Kuske et al., 2019) and supported by biomass estimates of bacteria and fungi using quantitative polymerase chain reaction (qPCR) of rRNA genes (Kuske et al., 2019; Manter et al., 2021) as described below.

2.4 | Quantitative PCR (qPCR) for structural and functional genes

The abundance of rRNA genes (copies g^{-1} dry soil) for bacteria and fungi and the phyla Acidobacteria, Verrucomicrobia, and Basidomycota were measured using qPCR (Stratagene Mx3005 real-time PCR) and Fast-Plus EvaGreen Master Mix with low ROX (Biotium) in triplicate for each sample DNA extract (Fierer et al., 2005). We attempted qPCR for Glomeromycota, but no protocol produced reliable data. Functional genes coding for C degradation (cellobiohydrolase I, beta-glucosidase), N fixation (dinitrogenase reductase, nifH), bacterial nitrification (ammonium monooxygenase, amoA), denitrification (copper containing nitrite reductase, nirK), and P mineralization (alkaline phosphatase, phoD) activities were enumerated in triplicate for all DNA extracts. For beta-glucosidase, SsoAdvanced Universal SYBR Green Supermix (BioRad) was used to achieve reliable results. Forward and reverse primer concentrations (300–1500 nM) were optimized for each target; primer names, their sequence, optimized concentrations, and literature reference are provided in Table S3. Annealing temperatures for each target were optimized and complete thermocycler conditions are provided in Table S4. Template DNA concentrations were standardized to 10 ± 1 ng DNA per reaction. Data were reported as gene copies g^{-1} dry soil using standard curves constructed via serial dilution of plasmids containing target DNA sequences amplified from type strains (structural genes) or extracted DNA pooled from all samples (functional genes) and cloned into plasmids via *Escherichia coli* JM109 using the pGEM-T Vector System II (Promega). Target insert size was verified by gel electrophoreses and sequence was confirmed by Sanger sequencing (Iowa State University DNA Facility); purified plasmids were quantified using Picogreen and Lambda DNA standards. Triplicate eight-point plasmid standard curves and five no-template negative controls were included on each qPCR microplate. No sample measurements fell outside of the standard curve range and assay detection limits were <10^3 copies per reaction. Enumerations of phyla were expressed as a proportion of their domain (Bacteria) or kingdom (Fungi) to allow compositional comparisons across samples.

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2.5 | Basal and substrate-induced respiration

Field moist soils held at 4°C (<1 week) were mixed and sieved (4.75 mm) and used to measure respiration in sealed jars (Zibilske, 1994). Thirty grams dry weight equivalent soil was placed in small plastic cups with drainage holes, gently tamped to achieve 1 gram cm^{-3} bulk density and placed into sealable glass jars. Soil water content was standardized to 50%–60% water-filled pore space for all samples with addition of water. For basal respiration no substrate was added while glucose or phenol at 1 mg g^{-1} dry soil were added with the water as carbon substrates for substrate-induced respiration (SIR). Glucose is a labile substrate and representative of root exudate compounds; phenol is a relatively recalcitrant substrate and representative of soil organic matter components. Headspace samples were withdrawn through three layers of septa and CO_2 produced during static 48-h incubations at 22°C was measured by a gas chromatograph (Shimadzu GC-2014) equipped with a methanizer and flame ionization detector. Positive (known standard) and negative (ambient air) controls were used in each run and an eight-point standard curve used to calculate CO_2 concentrations. Data were reported as carbon mineralized (mg CO_2-C kg^{-1} dry soil) during the 48-h incubation.

2.6 | Statistical analyses

Response variable distributions were assessed in JMP Software, 15.0 (JMP, 2023) via the distribution analysis with normal quantile plots. Gene abundance distributions were log normal and were natural log-transformed prior to any univariate or multivariate analysis. Respiratory response distributions were Gaussian and no data transformation occurred prior to analyses. Proportional data between taxa or respiration assays were natural log-transformed prior to analysis. Univariate statistical models were fit using the GLIMMIX Procedure in SAS Version 9.4, SAS/STAT 15.1 2016 by SAS Institute Inc. (SAS, 2022). The mixed model encompassed the main fixed effect of grazing management (AMP, conventional), and the random effects of the paired locations and their interaction with management. Location is a random effect in this study because of the a priori design decision to include locations that represent the geographic range of the study and are the sources of variation to test our main hypothesis of mean differences between the effect of the management types. The experimental unit of the transect (smallest independent plot unit) within the catena and location setting was identified in the random statement. In a few of the univariate mixed models, the variance component estimates of the random effects were negative. These variance component estimates were allowed to remain negative using the NOBOUND option. According to Stroup et al. (2018), this is the recommended procedure for better control over Type I error. The Kenward–Roger denominator degrees of freedom method was used because of the small-sample size approximation. LSMEANS and standard errors of the main management effect were calculated using the LSMEANS statement. Back-transformations of the LSMEANS and standard errors were calculated as necessary to report valuation in the original response range. Exact probabilities of management effects were reported; differences with $p < 0.05$ were considered significant.

A discriminant function (canonical) model was fit using JMP 15.0 Discriminant Analysis using the response variables evaluated by univariate statistical methodology. These 19 response variables included directly measured quantities and proportions expressed between two measured quantities. Grazing management (Conventional, AMP) was the grouping variable. Canonical details were calculated from the overall pooled within-group covariance matrix.

3 | RESULTS AND DISCUSSION

3.1 | Soil microbial community biomass

The total amount of DNA per gram dry soil, measured as a surrogate for soil microbial biomass (Kuske et al., 2019), was 32% greater ($p = 0.012$) under AMP compared to conventional management (Table 2). Analysis of variance main effects statistics are provided for all response variables in Table S5.

The abundance of rRNA genes was used to estimate the relative biomass of bacteria and fungi and to calculate fungal/bacterial (F:B) ratios, a potential indicator of land management (Kuske et al., 2019; Manter et al., 2021). In support of the total DNA assay results, there were 37% greater bacterial ($p = 0.001$) and 33% greater fungal ($p = 0.009$) ribosomal gene copies under AMP compared to conventional management (Table 2). The elevated amount of soil microbial biomass we observed under AMP management is consistent with greater amounts of soil C and N stocks, vegetation biomass, and water infiltration reported in these AMP-managed farms compared to their conventionally managed counterparts (Apfelbaum et al., 2022; Mosier et al., 2021). In studies of continuous livestock grazing, soil microbial biomass often increases with low to moderate grazing pressure, but declines with high-intensity continuous grazing (Bardgett et al., 1997, 2001; Ingram et al., 2008; Xun et al., 2018; Zhao et al., 2017). In two previous studies of grazed grasslands, multi-paddock cattle grazing resulted in greater soil microbial biomass compared to continuous grazing (Kleppel, 2019; Teague et al., 2011). Increased soil microbial biomass and soil C stocks in the top 30 cm of soil in well-managed, rotationally grazed grasslands in

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TABLE 2 Soil microbial biomass estimated by total DNA and numbers of ribosomal gene abundances per gram of soil according to grazing management practice.

Response variable	Grazing management		Management effect[a], Pr > F
	Conventional, mean ± (SE)	AMP, mean ± (SE)	
DNA (ng g^{-1})	6.05×10^3 (4.64×10^2)	8.00×10^3 (6.13×10^2)	0.012
Bacteria (gene copies g^{-1})	9.46×10^9 (5.87×10^8)	1.30×10^{10} (8.04×10^8)	0.001
Fungi (gene copies g^{-1})	5.25×10^7 (3.94×10^6)	7.00×10^7 (5.26×10^6)	0.009

Note: Data are means ± 1 standard error (SE) of samples collected from the five farms under each grazing management.
Abbreviation: AMP, adaptive-multipaddock grazing.
[a]Differences between grazing management for the mean of each response variable were considered significant at $p < 0.05$.

TABLE 3 Relative proportion of taxa enumerated by qPCR of ribosomal genes in soils.

Response variable	Grazing management		Management effect[a], Pr > F
	Conventional, mean ± (SE)	AMP, mean ± (SE)	
Fungi:Bacteria	0.0055 (0.0005)	0.0054 (0.0005)	0.838
Acidobacteria:Bacteria	0.0719 (0.0043)	0.0859 (0.0051)	0.040
Verrucomicrobia:Bacteria	0.0715 (0.0085)	0.0491 (0.0058)	0.028
Basidomycota:Fungi	0.0233 (0.0044)	0.0077 (0.0014)	<0.001

Note: Data are means ± 1 standard error (SE) of samples collected from the five farms under each grazing management.
Abbreviation: AMP, adaptive-multipaddock grazing.
[a]Differences between grazing management for the mean of each response variable were considered significant at $p < 0.05$.

comparison to other crop and forage production systems have been reported (Rui et al., 2022), although this outcome is surely dependent on edaphoclimatic conditions, prior land use, and vegetation assemblies (Bai & Cotrufo, 2022). While soil microbial biomass represents a relatively small fraction of total soil C, their activities transform plant exudates and residues into soil organic matter and more microbial biomass. The microbial biomass itself represents the feedstock for soil microbial necromass which interacts with soil minerals to form stabilized soil organic matter (Bai & Cotrufo, 2022). Greater (25%) mineral-stabilized soil organic matter has been reported in these same AMP-managed farms compared to the conventionally managed farms (Mosier et al., 2021).

3.2 | Soil microbial taxa indicators

There was no difference ($p = 0.838$) between grazing management with respect to F:B ratios which are a coarse indicator of community structure and viewed as being sensitive to land management (Table 3).

Elevated F:B ratios are often associated with land management practices that retain soil C and N and provide ecosystem services (de Vries et al., 2012, 2013; Six et al., 2006), but this association is altered by soil moisture (Frey et al., 1999), fertilization (Bardgett & McAlister, 1999), and plant assemblages (Hui et al., 2017; Wardle et al., 2004). High-intensity continuous grazing has been linked with lower F:B ratios (Bardgett et al., 1997, 2001; Xun et al., 2018), although soil and climatic conditions factor into these relationships (Bardgett & Wardle, 2003). Multi-paddock cattle grazing resulted in greater soil F:B ratios compared to continuous grazing in grasslands of upstate New York (Kleppel, 2019) and northern Texas (Teague et al., 2011). In the current study, bacteria were equally elevated with fungi under AMP grazing, possibly due to increased labile root exudates from the greater forage biomass reported at these farms (Apfelbaum et al., 2022).

Additional qPCR protocols were used to measure the number of rRNA gene copies for two bacterial phyla and two fungal phyla to assess further potential differences in community structure (Fierer et al., 2005). Acidobacteria rRNA gene numbers ($p < 0.001$; data not shown) and their proportion of total bacteria ($p = 0.040$; Table 3) were 64% and 37%, respectively, greater under AMP compared to conventional management. Acidobacteria is a diverse phylum with large genomes capable of versatile transformations of organic carbon, nitrogen, and sulfur; production of plant growth-promoting secondary metabolites, and production of extracellular polysaccharides involved in soil aggregation (Kalam et al., 2020). Wessen et al. (2010) reported Acidobacteria relative abundances measured by qPCR were influenced by soil fertility treatments, suggesting their potential as indicator land management, which is consistent with our finding. However, Eichorst et al. (2011) found subdivisions of Acidobacteria responded differently to land management and subdivision 4 was negatively correlated with SOC concentrations. In the current study, differences in the proportion

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of Acidobacteria with grazing management reflect community compositional changes, but given the high diversity within Acidobacteria, it is difficult to assign an ecological significance to this result.

Verrucomicrobia are abundant in soil, particularly in grasslands (Bergmann et al., 2011; Fierer et al., 2013). There was no difference ($p = 0.774$) in total abundances of Verrucomicrobia (data not shown) between grazing management practices, however, there was a 46% greater Verrucomicrobia:Bacteria proportion ($p = 0.028$, Table 3) under conventional compared to AMP management. Prior research has demonstrated high proportions of Verrucomicrobia sequences in undisturbed tall grass prairie remnants along a north–south transect across the central United States (Fierer et al., 2013) and their potential use as an indicator of grassland restoration in comparison to arable land has been suggested (Armbruster et al., 2021). Fierer et al. (2013) and Armbruster et al. (2021) targeted grasslands that were unimproved for grazing, in some cases for more than 100 years, a long-term land use history that differs substantially with more intensively grazed grasslands. In a controlled study of cattle grazing intensity, Verrucomicrobia abundances in soil were reduced with increasing continuous grazing intensity (Xun et al., 2018). In two studies using the grazing exclusion approach, Verrucomicrobia abundances in grassland soils were reduced by livestock grazing (Wang et al., 2023; Zhang & Fu, 2021). In our study, increased labile root exudates from the greater forage biomass (Apfelbaum et al., 2022) and 13% greater SOC stocks (Mosier et al., 2021) reported under AMP management may have reduced abundances of the oligotrophic Verrucomicrobia compared to conventional management.

There were 127% greater ($p = 0.013$) gene copy numbers (data not shown) of Basidiomycota and their proportion of fungi was 203% greater ($p < 0.001$) under conventional compared to AMP management (Table 3). Basidiomycota are considered to be sensitive to environmental perturbation and may be negatively affected by intensive grazing (Xun et al., 2018). Known as wood decomposers, Basidiomycota form large mycelia networks and are often dominant in forest soils (Buée et al., 2009), and the fields sampled on several conventionally managed farms were in close proximity to woodlands (locations 1, 4, and 5; Table 1) compared to their AMP counterparts. Increases in Basidiomycota have also been linked to lowered pH due to grazing management practices (Eldridge et al., 2017), and the average pH (pH 5.6) under conventional management was lower than under AMP management (pH 5.9) (Mosier et al., 2022).

Obligate plant symbiotic Glomeromycota frequently supply an array of benefits to their plant hosts and are considered prime candidates as bioindicators in agricultural production systems (Gottshall et al., 2017). As such, a defensible and efficient method to estimate Glomeromycota soil biomass is in demand given the shortcomings of currently used methods (Lehman et al., 2019). Unfortunately, Glomeromycota are present in low relative abundances in soil and preferential amplification often requires nested PCR protocols (Lee et al., 2008), which had to be avoided for qPCR. Successful primer sets that include most orders yet avoid nonspecific binding to other fungi or eukaryotes tend to be longer than requirements for qPCR. Despite extensive trials with different primer pairs and amplification conditions using axenic cultures of target and non-target organisms, no reproducible qPCR protocol emerged that overcame these challenges (data not shown).

3.3 | Soil microbial community functional potential

The numbers of six functional genes associated with C, N, and P cycling were measured with qPCR. There was no difference ($p = 0.757$) between grazing management for beta-glucosidase, a cellulase which catalyzes the terminal step of cellulose degradation by converting cellobiose to glucose, commonly considered the rate-limiting step for cellulose degradation (Table 4).

There was little response to the beta-glucosidase assay overall and optimizing this assay was difficult; an eventual change in qPCR kits achieved reproducible, but relatively low responses. Other researchers have used this beta-glucosidase protocol as a decomposition indicator with better success, although with different soils (Pérez-Guzmán et al., 2021). However, under adaptive-multipaddock grazing (AMP) management there were numerically greater (119%), but not statistically significant ($p = 0.084$) numbers of cellobiohydrolase genes compared to conventional management (Table 4). Cellobiohydrolase is an exocellulose that catalyzes the cleavage of cellobiose from the ends of cellulose polymers. Both cellulases are widely distributed in fungal and bacterial taxa and the elevated levels of cellobiohydrolases under AMP management are consistent with the greater microbial biomass found under AMP compared to conventional management. Genes for N fixation, nitrification, denitrification, and organic P hydrolysis are less widely distributed among soil microbial taxa (Nelson et al., 2016; Ragot et al., 2016) compared to cellulases. The number of nitrogen fixation genes (nifH) was numerically greater (19%) under AMP compared to conventional management but there was considerable variability among the AMP farms ($p = 0.540$, Table 4). Nitrification (amoA) and denitrification (nirK) genes were 386% and 113%, respectively, more numerous ($p < 0.001$) under AMP compared to conventional management (Table 4). Nitrogen is considered the limiting nutrient for terrestrial primary production, and ammonium oxidation is a critical biotransformation that increases the availability of N to plants. Bacterial nitrification and denitrification genes have been shown to respond to grassland grazing. Bacterial amoA genes increased

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TABLE 4 Soil microbial community functional potential assessed by the numbers of functional genes involved in carbon, nitrogen, and phosphorus cycling.

Response variable	Grazing management		Management effect[a], Pr > F
	Conventional, gene copies gram^{-1}, mean ± (SE)	AMP, gene copies gram^{-1}, mean ± (SE)	
Beta-glucosidase	7.67×10^4 (9.12×10^3)	7.28×10^4 (8.66×10^3)	0.757
Cellobiohydrolase	1.69×10^5 (5.33×10^4)	3.70×10^5 (1.17×10^5)	0.084
nifH	5.05×10^6 (5.69×10^5)	5.99×10^6 (1.17×10^6)	0.540
amoA	3.23×10^4 (8.10×10^3)	1.57×10^5 (3.93×10^4)	<0.001
nirK	1.57×10^6 (1.67×10^5)	3.35×10^6 (3.42×10^5)	<0.001
phoD	1.34×10^7 (5.85×10^6)	5.71×10^7 (2.48×10^7)	0.022

Note: Data are means ± 1 standard error (SE) of samples collected from the five farms under each grazing management.
[a]Differences between grazing management for the mean of each response variable were considered significant at $p < 0.05$.

in abundance with sheep grazing intensity (Pan et al., 2018) while another study reported amoA gene numbers decreased with land degradation by livestock grazing (Zhang et al., 2022). Two studies found that nirK genes became more abundant with livestock grazing intensity (Zhang et al., 2022; Zhong et al., 2016) and Zhong et al. (2016) reported concurrence between nirK numbers and independent measures of denitrification. Alkaline phosphatase (phoD) hydrolyzes organic phosphoesters and mobilizes P to be used by microorganisms and plants. There were 326% greater ($p = 0.022$) numbers of phoD gene copies under AMP compared to conventional management indicating an increased potential for mobilizing P from soil organic matter (Table 4). The results of functional gene analyses indicate that soils under AMP management were enriched in soil microorganisms that perform N and P cycling activities and make these nutrients bioavailable to vegetation. These results are consistent with the greater soil organic C and N stocks (Mosier et al., 2021) and forage production (Apfelbaum et al., 2022) reported under AMP management compared to conventional management at these farms.

3.4 | Basal and substrate-induced soil microbial respiratory activities

There was a significant effect of grazing management on soil basal respiration with AMP having 55% greater ($p = 0.004$) basal respiration than conventional management (Table 5).

Greater basal respiration per gram of dry soil means either microorganisms that are more active, or simply more microorganisms. Our finding of elevated microbial biomass under AMP management supports that latter explanation. Increased activities would be expected as a consequence of increased plant root exudates and residues from the larger standing crop of vegetation under AMP grazing compared to conventional management reported at these farms (Apfelbaum et al., 2022). In studies of continuous grazing, light grazing may increase soil respiration while heavy grazing pressure decreases respiration, probably due to greater soil bulk densities and less root biomass (Ingram et al., 2008; Patra et al., 2005; Zhao et al., 2017).

The SIR assay used the identical procedure as basal respiration, with the difference being the addition of 1 mg g^{-1} soil substrate with the water used to normalize soil water content. This substrate concentration is higher than in ambient soil, but far less than the saturating substrate concentration used with SIR procedures for estimating biomass (Anderson & Domsch, 1978). Moreover, the SIR assay did not use extensive soil preconditioning, small amounts of soil (i.e., 1 g), soil slurries, agitation, or a short incubation (e.g., 6 h) used with SIR procedures to estimate biomass (Anderson & Domsch, 1978; West & Sparling, 1986). Both management practices displayed increased respiration with the addition of glucose, but there was no significant effect of grazing management on glucose-induced respiration (Table 5). However, AMP had 38% greater ($p = 0.008$) phenol-induced respiration than conventional management (Table 5). This was interpreted as a difference in soil C processing (Frey et al., 2013), presumably reflecting a larger, more active, and metabolically versatile biomass with the ability to rapidly produce extracellular enzymes necessary to degrade complex substrates. Ratios of the respiratory activities were calculated to assess carbon substrate limitations on microbial respiration (Cheng et al., 1996; Parkinson & Coleman, 1991). Conventional management had 44% greater ratios of glucose-induced:basal activities ($p = 0.009$) and 7% greater glucose:phenol substrate-induced activities ($p = 0.010$; Table 5) than AMP management which indicate strong limitation of C mineralization activities by the amount of labile C such as root exudates. This explanation is consistent with the decreased vegetation biomass reported under conventional compared to AMP management at these same farms (Apfelbaum et al., 2022). Greater labile C limitation reduces soil C processing and the formation of microbial biomass, which is the predecessor of microbial necromass and the formation of stabilized soil organic matter.

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TABLE 5 Soil basal and substrate-induced respiration (SIR) with glucose and phenol as substrates and ratios between these measures.

Response variable	Grazing management		Management effect[a], Pr > F
	Conventional, mean ± (SE)	AMP, mean ± (SE)	
Basal respiration (mg C kg^{-1})	25.54 (3.37)	39.64 (3.37)	0.004
Glucose SIR (mg C kg^{-1})	145.42 (5.53)	148.42 (5.52)	0.688
Phenol SIR (mg C kg^{-1})	35.69 (3.56)	49.42 (3.55)	0.008
Glucose:Basal	6.36 (0.61)	4.41 (0.42)	0.009
Phenol:Basal	1.43 (0.06)	1.34 (0.04)	0.308
Glucose:Phenol	4.47 (0.36)	3.29 (0.27)	0.010

Note: Data are means ± 1 standard error (SE) of samples collected from the five farms under each grazing management.
Abbreviation: AMP, adaptive-multipaddock grazing.
[a]Differences between grazing management for the mean of each response variable were considered significant at $p < 0.05$.

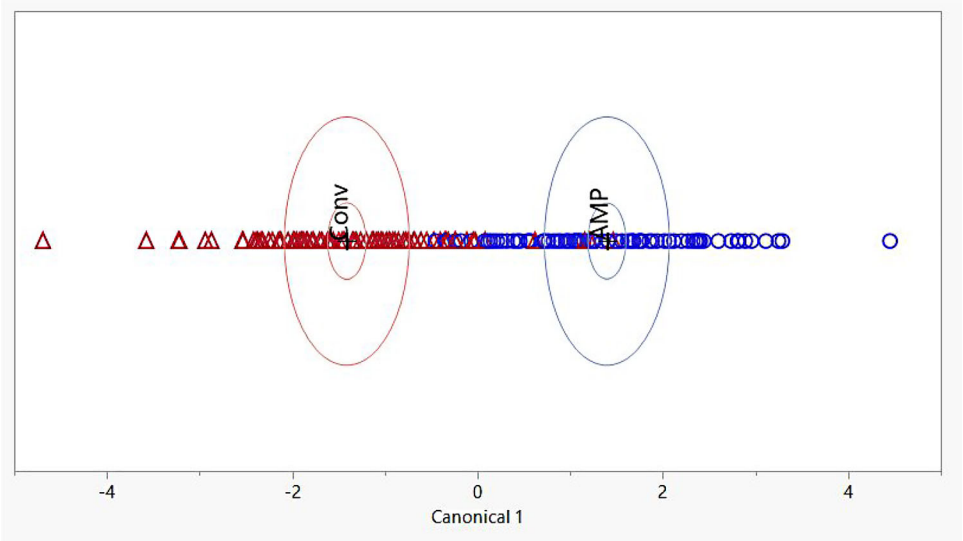

FIGURE 1 Management classification of the 179 soil samples based on their responses to the 19 measured biological response variables using discriminant function analysis. Red triangles represent soil samples under conventional management and blue circles those from AMP management. The small and large ovals are the 95% mean confidence limit and the normal 50% distribution, respectively. The mean value of the scores on canonical axis 1 for conventional management was −1.412 and AMP management was 1.396; the canonical separation of these two management groups was signficant (<0.0001, Wilks' Lambda). AMP, adaptive multi-paddock grazing.

TABLE 6 Canonical details of discriminant function analysis calculated from the overall pooled within-group covariance matrix.

Eigenvalue	Percent	Cum percent	Canonical Corr	Wilks' Lambda	Exact F	NumDF	DenDF	Prob > F
1.9929	100.0	100.0	0.8160	0.3341	16.6774	19	159	<0.0001

3.5 | Distinguishing site and grazing management by discriminant function analyses of all biological data

Discriminant function analysis using the 19 biological response variables listed in Tables 2–5 effectively classified ($p < 0.001$. Wilks' Lambda) the soil samples according to the two management practices (Figure 1, Table 6).

Of the 179 soil samples (one sample had missing data for respiratory measures and was removed prior to analysis) collected from the 10 farms, 94% were classified within the proper management practice based on their response to the 19 variables. Therefore, given an unknown soil sample from any farm, analysis of these 19 response variables would allow the unknown soil sample to be assigned to the correct grazing management practice 94 times out of 100.

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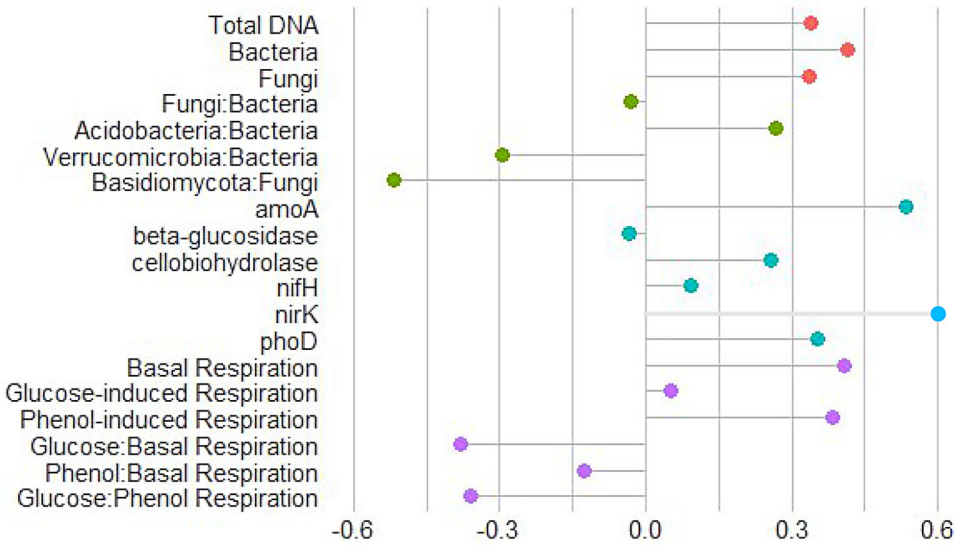

FIGURE 2 Total canonical loadings for canonical axis 1 of discriminant function analysis presented in Figure 1. Positive loadings are associated with AMP grazing management and negative loadings are associated with conventional grazing management. Biomass-related measures are in orange, community structure in green, community potential function in blue, and carbon mineralization activities in purple. amoA, ammonium monooxygenase; AMP, adaptive multi-paddock grazing; nifH, dinitrogenase reductase; nirK, copper containing nitrite reductase, phoD, alkaline phosphatase.

The total canonical loadings from the discriminant function analysis (Figure 2) reflect the strength of each response variable in properly classifying the soils by management practice. Microbial biomass indicators, genes involved in carbon and nutrient transformation, and basal and phenol-induced respiration were positively associated with AMP management. A minority of response variables were positively associated with CG management including Basidomycota and Verrucomicrobia relative abundances and glucose-induced respiration relative to basal or phenol-induced respiration which indicates labile carbon limitation. The canonical loadings mirror the univariate analyses conducted, but together combine to effectively distinguish the management practices.

Discriminant function analyses clearly illustrated that AMP grazing management resulted in distinctive soil microbial communities characterized by elevated biomass, C and nutrient cycling genes, basal respiration, and reduced labile carbon limitation of C mineralization compared to conventional management. The soil microbial properties under AMP management were associated with greater soil C and N stocks (Mosier et al., 2021), standing crop forage biomass (Apfelbaum et al., 2022), and cattle stocking rates (Table S1) under AMP compared to conventional management at these same farms. The biological properties that were strongly associated with grazing management (Figure 2) represent candidate dynamic indicators for assessing grazing land soil health at other sites, providing soil sample preservation requirements are met for these assays.

4 | CONCLUSIONS

Cattle grazing management practices differentially influenced soil microbial properties at pairs of farms at five locations arranged on north–south transect in the southeastern United States. On average of these five locations, there was greater soil microbial biomass, numbers of genes involved in carbon and nutrient cycling, and carbon mineralization activities under AMP compared to conventional management. Carbon mineralization under conventional management was strongly limited by access to labile carbon substrates. A large and active soil microbial community under AMP management should maintain access to nutrients for plants and stimulate stable soil organic matter formation which is corroborated by previously published vegetation and SOC data collected by other researchers at these same farms. Classification of soils according to their management using 19 biological properties was successful and identified candidate measures for grazing land assessments including estimates of microbial biomass, respiratory activities, and genes involved with nitrogen and phosphorous cycling. Replicated farm-scale studies of the response of soil microbiological properties to grazing management across a range of edaphoclimatic conditions are needed to fully understand the relationships between grazing management and soil microorganisms.

AUTHOR CONTRIBUTIONS

Laura J. White: Data curation; formal analysis; investigation; methodology; validation; writing—original draft;

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writing—review and editing. **Kathleen M. Yeater**: Formal analysis; writing—review and editing. **Richard Michael Lehman**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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