



Conversion of open pasture to hardwood silvopasture enhanced soil health of an ultisol

Sanjok Poudel · Sangeeta Bansal ·
Swarup Podder · Bidur Paneru · Sangita Karki ·
John Fike · Sandeep Kumar

Received: 19 October 2021 / Accepted: 7 October 2022 / Published online: 11 October 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract Black walnut (*Juglans nigra*) and honeylocust (*Gleditsia triacanthos*) have gained particular interest for use in silvopastures and other temperate agroforestry systems. However, measures of soil health indicators in these deciduous hardwood silvopastures over time have received limited attention. This study assessed soil health indicators in 25-year-old black walnut- (BSP) and honeylocust-based silvopastures (HSP) and compared with those from adjacent open pasture (OP) systems. Soil samples collected from 0 to 10 cm depth were analyzed for soil organic matter (OM), carbon and nitrogen fractions,

glomalin-related soil protein, soil enzymatic activities, and microbial community structure. The soil OM content in HSP (64 g kg^{-1}) was greater ($p < 0.05$) than BSP and OP (55 g kg^{-1}). The BSP soils had about 45% and 52% more ($p < 0.05$) microbial biomass carbon and nitrogen than OP soils. The HSP soils had 60% greater ($p < 0.05$) microbial biomass nitrogen than OP soils. The BSP supported greater ($p < 0.05$) β -glucosidase and urease activities than the HSP and OP treatments, while greater ($p < 0.05$) β -glucosidase activity was measured in HSP than OP. Total phospholipid-derived fatty acid and actinomycetes abundance were greater ($p < 0.001$) in OP compared to BSP and HSP while the arbuscular mycorrhiza fungi abundance was greater ($p < 0.05$) in OP than in HSP. These findings indicate that black walnut and honeylocust trees in the silvopastures with proper management can improve certain soil health parameters over time depending on tree characteristics and the age of the system.

S. Poudel (✉) · S. Podder · J. Fike
School of Plant and Environmental Sciences, Virginia
Polytechnic Institute and State University, Blacksburg,
VA 24061, USA
e-mail: sanjokp@vt.edu

S. Bansal
Department of Land, Air, and Water Resources, The
University of California, Davis, Davis, CA 95616, USA

B. Paneru
Department of Animal and Poultry Science, Virginia
Polytechnic Institute and State University, Blacksburg,
VA 24061, USA

S. Karki
Department of Biological Sciences, Virginia Polytechnic
Institute and State University, Blacksburg, VA 24061, USA

S. Kumar
National Institute of Food and Agriculture, United States
Department of Agriculture, Kansas City, MO 64105, USA

Keywords Black walnut · Honeylocust · Soil carbon · Soil enzymatic activities · PLFA

Introduction

Soil health as defined by Janzen et al. (2021) is “the viability of a soil in sustaining the socio-ecological functions of its enfolding land”. Healthy soil can enhance nutrient cycling and improve carbon

sequestration along with improving soil aggregate stability and overall biodiversity (USDA-NRCS 2014). Thus, enhancing and sustaining healthy soil can be considered a key aspect of agriculture sustainability. Silvopasture, the intentional integration of trees, livestock, and forages within a common management unit, is a form of agroforestry and a sustainable land management practice (Garrett et al. 2000). In addition to their potential production and economic values, silvopasture systems can perform several environmental services such as protecting water quality, conserving soil, and sequestering carbon (Garrett et al. 2000). Indeed, one analysis has suggested silvopasture management could be a “top ten” solution for carbon sequestration (Hawken 2017). Silvopastures have a substantial ability to sequester atmospheric carbon (C) and nitrogen (N) because they have both woody and herbaceous plant storage patterns active within the system (Sharrow and Ismail 2004).

Soil nutrient dynamics and overall soil health could be improved over time with silvopasture management given changes in above and belowground productivity, rooting depth and distribution, and the quantity and quality of organic matter inputs to the soil (Jackson et al. 2000). Trees within a silvopasture can minimize nutrient leaching, recycle nutrients, and enhance soil's physical properties (Schroth 1995). Additionally, trees can also increase C and N supply to the system and lower soil bulk density (Belsky et al. 1993; Haile et al 2008). Such inputs, along with a change in microclimate, may create better habitats for microbial growth thus improving soil microbial activity, diversity, and abundance (Cubillos et al. 2016). Soil enzyme activities in the silvopasture system can also be increased with the change in microbial communities (Karki et al. 2021; Stanley et al. 2008). Thus, establishing trees within a pasture might help improve overall soil health and increase the productivity of the system (Bosi et al. 2020; Pent 2020).

In temperate environments, hardwood trees such as black walnut (*Juglans nigra*) and honeylocust (*Gleditsia triacanthos*) have gained particular interest for agroforestry systems. Such species may be advantageous as they have relatively fast growth rates, open canopies, and phenologies that reduce competition with the growth of cool-season forages. Their root systems can stabilize soil and improve nutrient capture and recycling, their canopies provide shade to animals and produce nuts and fodder (e.g., leaves

and edible seed pods), and their timber has market value or potential for numerous farm uses (Bendfeldt et al. 2001). While the greater complexity of the silvopastures versus open pasture systems suggests they could significantly enhance soil health, little is known regarding the effects of silvopastoral management on soil health parameters—specifically when integrating black walnut and honeylocust trees. Knowledge of soil health indicators as influenced by the silvopasture system can help expand the theoretical basis for incorporating trees into pastures for improving overall soil health—and thus improving the sustainability of the system. To help fill this gap, we measured soil health indicators in mid-stage black walnut and honeylocust-based silvopastures and compared them to those from open pastures. We hypothesized that introducing temperate hardwood tree species such as black walnut and honeylocust within the silvopasture systems would alter soil organic carbon, soil pH, bulk density, microbial biomass, enzymatic activities, and microbial community structure compared with the adjacent open pasture system over the period of time.

Materials and methods

Study site, treatments, and soil sampling

This study was carried out at the Whitethorne Agroforestry Demonstration Center located at Virginia Tech's Kentland Farm in Blacksburg, Virginia (37° 12' 00.6" N 80° 34' 34.8" W). The study site lies in the Southern Appalachian Ridge and Valley Physiographic Province of Virginia. The site lies at 540 m above sea level with an average annual temperature of 10 °C. Average annual precipitation at the study site is 1500 mm. Predominant soils are Unison and Brad-dock fine, mixed, semiactive, mesic Typic Hapludults and Rayne fine-loamy, mixed, active, mesic Typic Hapludults. Three experimental treatments—open pastures (OP) and black walnut (BSP) and honeylocust (HSP) based silvopasture systems—are replicated three times randomly across the study site, and the area of each experimental unit is 0.27-ha.

Trees in the BSP and HSP silvopastures were planted into an existing cool-season pasture in 1995. A final thinning in 2012 left trees in a 12.2-m×12.2-m configuration. Orchardgrass (*Dactylis glomerata*) was the primary forage established in

1995, but the composition has changed over time and varies by treatment. Along with orchardgrass, systems contain tall fescue (*Schedonorus phoenix*), Kentucky bluegrass (*Poa pratensis*), nimblewill (*Muhlenbergia schreberi*), and red and white clovers (*Trifolium pratense* and *T. repens*) among others. Fescue is dominant in open and honeylocust systems.

Forage management has included periodic grazing with limited fertility inputs. Cattle (*Bos taurus*) grazed the site once or twice per growing season to take advantage of available forage, and sheep (*Ovis aries*) grazed the study site, generally in summer, for various research studies. Pastures have been managed with rotational stocking based on forage availability. Similar stocking rates have been used for all treatments when allowing livestock to graze. The site has been fertilized periodically, especially during late spring, before summer grazing, typically at a rate of 56–25–50 kg NPK per hectare.

Weed management has largely been limited to mechanical defoliation. In 2020, ProClova (florpyrauxifen + 2,4-D, Corteva Agriscience, Wilmington, DE) was applied at 4.3 L ha⁻¹ before summer grazing to control stickweed (*Verbesina occidentalis*) and other broadleaf weeds, including creeping thistle (*Cirsium arvense*) and milk thistle (*Silybum marianum*). Trees in the silvopastures have been trimmed periodically to remove the side branches (~2.5 to 5 m height) to maintain clear boles and keep branches away from field equipment. Existing tree density and tree basal area averages 91 stems ha⁻¹ and 6.1 m² ha⁻¹ in BSP and 104 stems ha⁻¹ and 4.2 m² ha⁻¹ in HSP.

Soil samples were collected in March 2021. Samples were collected from 8 random points within each experimental unit. At each sampling point, three soil cores were collected to 10-cm depth with a soil auger, then composited and thoroughly mixed. Sample points were kept about 2 m area from fence lines to minimize border effects and about 1 m from trees was maintained while collecting samples on the silvopasture plots. Soil samples were kept in zip-lock bags, labeled, stored in a cooler, and immediately returned to the lab. Soil samples for soil microbial biomass and enzyme activities analysis were stored in a refrigerator at 4 °C. A small portion of the soil sample collected was weighed and oven-dried at 105 °C for about 48 h to determine the moisture content. At the sampling points, another set of soil samples was collected for soil bulk density using a double-cylinder,

drop-hammer sampler with a core (AMS, Inc., American Falls, ID).

Soil physiochemical analyses

Samples were analyzed for soil pH (1:1, soil/water) with an AS-3000 Dual pH Analyser (Labfit Pty Ltd, Burswood, Western Australia) (Watson and Brown 1998). Soil organic matter (OM) was analyzed by the weight loss on ignition (LOI) method (Nelson and Sommers 1996). Soil acidity was estimated from the Mehlich soil buffer pH after conversion of all analytical results to cmol(+)/kg. Available soil phosphorus (P), potassium (K⁺), and calcium (Ca²⁺) were extracted using Mehlich 1 (0.05 N HCl in 0.025 N H₂SO₄) extracting solution and analyzed using an ICP (inductively coupled plasma atomic emission spectrometer). Soil nitrate–nitrogen (NO₃–N) and ammonium–nitrogen (NH₄–N) were determined by flow injection analysis (FIA) using a Lachat QuickChem 8000 Automated Ion Analyzer (Hach Company, Loveland, CO). The total C and total N in soil were analyzed using the dry combustion (Dumas) method using a CN elemental analyzer (Elementar Americas Inc., Ronkonkoma, NY).

Soil C and N fractions and microbial biomass

Water-extractable organic C and N fractions were analyzed using the protocol of Ghani et al. (2003). For cold-water extractable C and N (CWC and CWN), 3 g of soil were placed in 50-ml polypropylene centrifuge tubes and 30 ml of distilled water was added to the tube. The mixture was vortexed for 10 s, shaken on a rotatory shaker for 30 min at 40 rpm and centrifuged at 3000 rpm at 4 °C for 25 min to extract the CWC and CWN. For hot-water extractable C and N (HWC and HWN), 30 ml of distilled water was added to the remaining soil, placed in a hot water bath (80 °C) for 12 h and centrifuged at 3000 rpm for 25 min at 25 °C. The water-extractable C and N fractions were then analyzed in a TOC-L analyzer (Shimadzu Corporation, Columbia, MD). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform fumigation direct extraction method (Gregorich et al. 1990). Eight (8) g of soil was fumigated with alcohol-free chloroform in a desiccator for 24 h and extracted with 50 ml of 0.5 M K₂SO₄. For non-fumigated soil, 50 ml of 0.5 M K₂SO₄ were used

for extraction and both extracts were analyzed with the TOC-L analyzer. The MBC and MBN concentrations were calculated as the difference between C and N values from fumigation and non-fumigation analysis with a correction factor of 0.45 (Beck et al. 1997).

Glomalin-related soil protein (GRSP) and soil enzymatic activity

The glomalin-related soil protein (GRSP) was extracted using the protocol of Wright and Upadhyaya (1998) with slight modification. About 3 g of soil were mixed with 24 ml of 20 mM sodium citrate solution (pH 7.0) and shaken well in an orbital shaker to disperse the aggregates and mixed well for 5 min at 180 rpm. The samples were autoclaved for 30 min (121 °C, 15 psi) and then were allowed to cool to room temperature. Two (2) ml of the resulting slurry were placed into a microcentrifuge tube and centrifuged at 10,000×g for 15 min to pelletize the soil particles. GRSP values were standardized to total protein content using a standard colorimetric protein quantification assay (BCA).

Enzyme assays

Urease activity in soil was determined following the protocols of Kandeler and Gerber (1988). Five (5) g of soil was placed in each of three 50-ml incubation flasks and 2.5 ml of urea solution (720 mM) was added to the first two flasks (the third flask was used as a control) and 20 ml of borate buffer (0.1 M, pH 10) was added to all three flasks. All the flasks were parafilm-sealed and incubated at 37 °C for 2 h. The released ammonium was determined using a spectrophotometer at 660 nm. The β -glucosidase activity in soil was determined following the protocol of Eivazi and Tabatabai (1988). One (1) g of soil was mixed with 0.2 ml toluene, 4 ml of modified universal buffer (MUB, pH 6.0), and 1 ml of 50 mM *p*-nitrophenyl- β -D-glucoside (PNG) solution and incubated for 1 h. After incubation, 1 ml of 0.5 M CaCl_2 and 4 ml of 0.1 M THAM buffer (pH 12.0) were added to the flask to end the reaction. The soil suspension was filtered and the filtrate was read at 405 nm using a spectrophotometer for yellow color intensity. Each assay also included a control sample by using a similar procedure along with the addition of substrate PNG solution after adding THAM buffer. Soil acid

phosphatase activity was determined following the methods of Tabatabai and Bremner (1972) and Eivazi and Tabatabai (1977). Briefly, 1 g soil was mixed with 0.25 ml toluene (0.25 ml), 4 ml MUB buffer (pH 6.5), and 1 ml of *p*-nitrophenyl-phosphate solution (0.05 M) and incubated at 37 °C for 1 h. After incubation, 1 ml of 0.5 M calcium chloride (CaCl_2) and 4 ml of sodium hydroxide (NaOH; 0.5 M) was added to terminate the reaction and the soil suspension was filtered. The filtrate was read at 400–410 nm using a spectrophotometer for yellow color intensity. All enzyme activities were expressed in $\text{nmol g}^{-1} \text{h}^{-1}$.

Microbial community structure

The microbial community structure in soil samples was determined using a phospholipid fatty acids (PLFA) analysis (Clapperton et al. 2005). Briefly, 2 g of lyophilized soil was mixed with 9.5 ml dichloromethane (DMC):methanol (MeOH):citrate buffer (1:2:0.8 v/v) extraction solution to extract total soil lipids. The extracted solution was passed through a solid-phase silica column to separate phospholipids from other lipids. Fatty acid methyl esters (FAMES) extracted were assessed using an Agilent 2030-GC equipped with a CP-7693 auto-sampler and a flame ionization detector (FID).

Statistical analysis

All statistical procedures were carried out in RStudio V1.4.1106. A one-way analysis of variance (ANOVA) test was carried out to test for the effect of treatments on soil physiochemical properties, soil C and N fractions, enzymatic activities, and microbial community composition. Similarly, least-squares means and Tukey's adjusted differences of the means were also estimated. Differences were considered statistically significant at $p < 0.05$ and were considered a trend at $p < 0.10$.

Results

Soil physiochemical properties

Treatments did not impact soil bulk density ($p = 0.6799$); values within BSP, HSP, and OP were 1.27, 1.30, and 1.37 g cm^{-3} respectively. Soil pH

did not differ among pasture management treatments ($p=0.4695$; Table 1). The HSP soils tended ($p<0.1$) to be less acidic and have greater base saturation and Ca^{2+} than OP soils. Soil P, Mg, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ did not differ ($p\geq 0.1906$) among the treatments. The HSP soils had greater ($p<0.05$) K^+ content than OP

Table 1 Soil physicochemical properties under black walnut (*Juglans nigra*) and honeylocust (*Gleditsia triacanthos*) based silvopastures and open pasture systems

Parameters	Treatments [†]				<i>p</i> value
	BSP	HSP	OP	SE	
BD (g cm^{-3})	1.3	1.3	1.4	0.08	0.6799
pH	5.9	5.9	5.6	0.14	0.4695
Acidity (%)	14.8	14.0	22.8	2.52	0.0880
$\text{BS}^{\ddagger}$ (%)	85.2	85.9	77.2	2.52	0.0880
P (mg kg^{-1})	14.3	15.3	16.7	3.42	0.8912
K (mg kg^{-1})	167.3 ^{ab}	184.7 ^{a*}	133.3 ^b	10.35	0.0329
Ca (mg kg^{-1})	942.3	992.3	728.3	71.32	0.0834
$\text{NO}_3\text{-N}$ (mg kg^{-1})	4.5	4.8	3.5	0.40	0.3702
$\text{NH}_4\text{-N}$ (mg kg^{-1})	0.4	0.4	0.3	0.02	0.2160

^{abc}Means with different superscripts in a row differ ($*p<0.05$)

[†]BSP black walnut silvopasture, HSP honeylocust silvopasture, OP open pasture, SE standard error of means

[‡]BS base saturation, P phosphorus, K potassium, Ca calcium, BD bulk density

soils. The soil OM content in BSP, HSP, and OP soils was 55, 64, and 55 g kg^{-1} respectively with a greater ($p<0.05$) soil OM content in HSP compared to BSP and OP soils (Fig. 1). HSP soils also had greater ($p<0.05$) total C than OP soils; however, total C in BSP soils was intermediate and did not differ from HSP and OP treatments (Fig. 1). The HSP soils tended ($p<0.1$) to have greater total N than OP soils. Silvopasture soils had a greater ($p<0.001$) C:N ratio than OP soils.

Glomalin-related soil protein (GRSP) and soil enzymatic activity

Levels of GRSP tended ($p<0.06$) to be greater in HSP than in OP soils (4.5, 5.2, and 4.3 mg g^{-1} in BSP, HSP, and OP respectively; Fig. 2). Soil enzymatic activity was generally greater ($p<0.05$) in BSP than in HSP and OP soils. The silvopasture soils had greater ($p<0.05$) β -glucosidase activity than OP soils, but acid phosphatase activity did not differ among treatments. BSP soils also had greater ($p<0.05$) urease activity than OP and HSP soils; however, urease activity did not differ among HSP and OP treatments.

Fig. 1 Soil organic matter, total carbon, total nitrogen, and C:N ratio (mean $\pm$ SE) under black walnut (*Juglans nigra*; BSP) and honeylocust (*Gleditsia triacanthos*, HSP) based silvopastures and open pasture (OP) systems. (Tukey HSD; $*p<0.05$, $**p<0.01$)

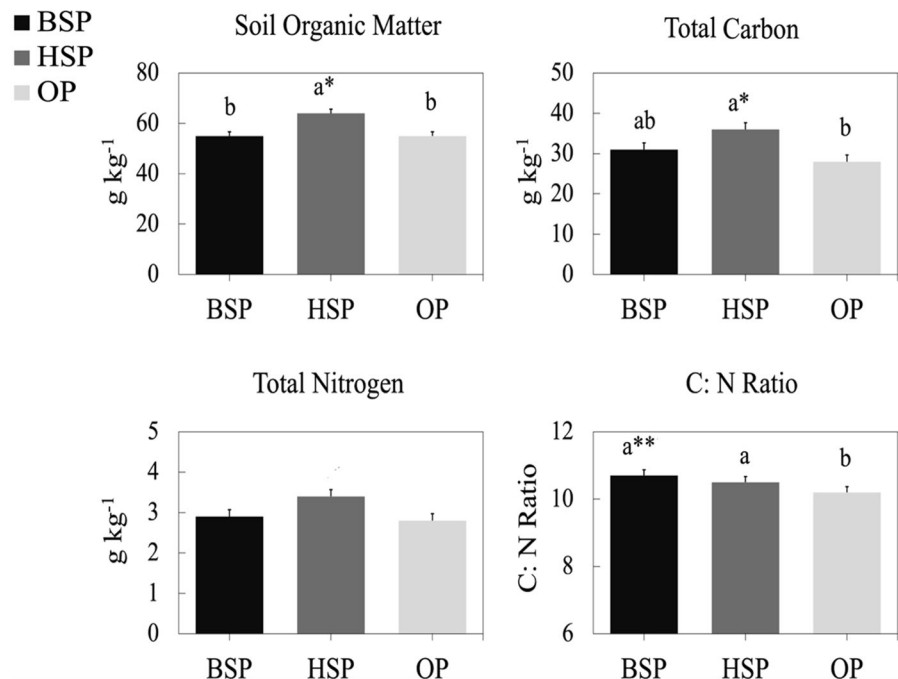
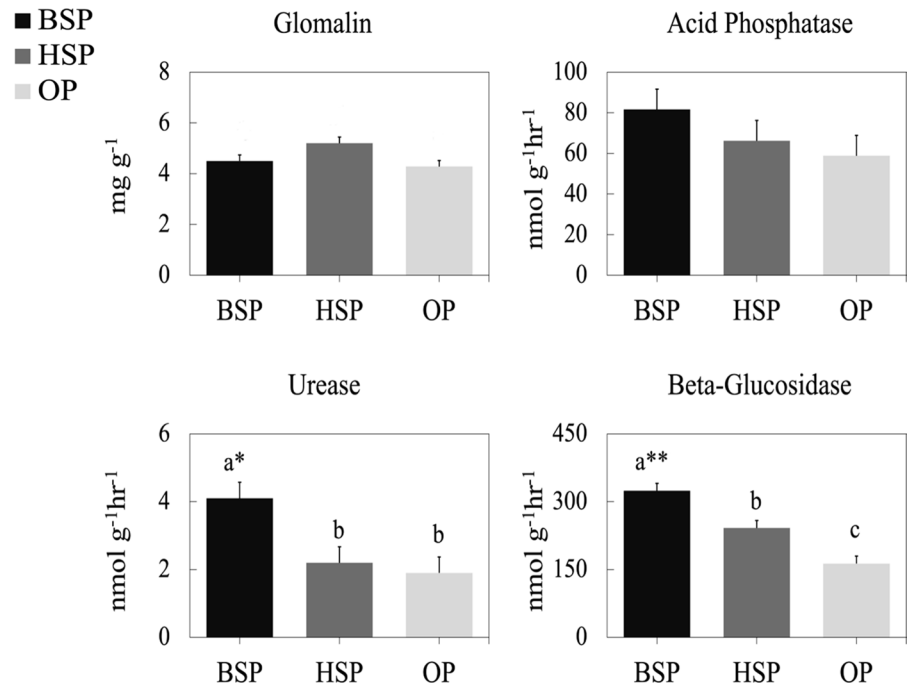


Fig. 2 Glomalin-related soil protein (GRSP) and soil enzymatic activities (mean $\pm$ SE) under black walnut (*Juglans nigra*; BSP) and honeylocust (*Gleditsia triacanthos*, HSP) based silvopastures and open pasture (OP) systems. (Tukey HSD; * $p < 0.05$, ** $p < 0.01$)



Soil carbon and nitrogen fractions

The soil labile C and N fractions were greater in silvopasture treatments compared to the OP (Table 2). The BSP soils had about 45% and 52% more ($p < 0.05$) MBC and MBN than OP soils. The HSP soils had intermediate MBC levels (not different among treatments) but 60% greater ($p < 0.05$) MBN than OP. The CWC, HWC, and HWN did not differ among treatments while HSP and OP soils tended ($p < 0.1$) towards greater CWN content compared to BSP soils.

Soil microbial community structure

Systems had diverse and distinctive ecological niches for soil microbial communities. The OP soils had about fourfold more ($p < 0.001$) total PLFA than BSP and HSP soils (Table 3). Conversely, arbuscular mycorrhizal fungi (AMF) abundance was greater ($p < 0.05$) in OP soils compared to BSP soils. Actinomycetes abundance was much greater ($p < 0.001$) in OP soils than in BSP and HSP soils. No differences among treatments were measured for gram-positive (GP) and gram-negative (GN) bacteria, nor could differences among treatments be discerned for total fungi and total bacterial abundance.

Table 2 Cold and hot water-soluble organic carbon (CWC and HWC), cold and hot water-soluble organic nitrogen (CWN and HWN), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) under black walnut (*Juglans nigra*) and honeylocust (*Gleditsia triacanthos*) based silvopastures and open pasture systems

Parameters [‡]	Treatments [†]				<i>p</i> value
	BSP	HSP	OP	SE	
	$\mu\text{g g soil}^{-1}$				
MBC	1727.1 ^{a*}	1538.9 ^{ab}	1183.1 ^b	116.29	0.0258
MBN	206.5 ^{a***}	217.7 ^{a***}	135.7 ^b	15.26	0.0085
CWC	22.2	24.1	18.8	1.96	0.2072
HWC	148.0	170.0	151.5	12.84	0.4598
CWN	2.0	3.0	3.1	0.29	0.0667
HWN	18.5	20.1	17.2	1.31	0.3409

^{abc}Means with different superscripts in a row differ (* $p < 0.05$, ** $p < 0.01$)

[†]BSP black walnut silvopasture, HSP honeylocust silvopasture, OP open pasture, SEM standard error of means

[‡]MBC microbial biomass carbon, MBN microbial biomass nitrogen, CWC cold water-soluble organic carbon, HWC hot water-soluble organic carbon, CWN cold water-soluble organic nitrogen, HWN hot water-soluble organic nitrogen

Table 3 Total PLFA (phospholipid fatty acid), total bacterial, actinomycetes, Gram-negative (–ve) and Gram-positive (+ve) bacterial, total fungi, Arbuscular Mycorrhizal (AM) fungi under black walnut (*Juglans nigra*) and honeylocust (*Gleditsia triacanthos*) based silvopastures and open pasture systems

Parameters	Treatments [†]				<i>p</i> value
	BSP	HSP	OP	SE	
	nmol g soil ^{–1}				
PLFA [‡]	7.2 ^b	8.5 ^b	33.2 ^{a***}	1.38	<0.001
Total fungi	0.6	0.4	0.5	0.09	0.5471
Total bacteria	5.6	4.1	2.9	0.94	0.1931
AMF	0.6 ^b	6.8 ^{ab}	12.9 ^{a*}	2.57	0.0252
Actinomycetes	1.0 ^b	3.9 ^b	29.7 ^{a***}	1.21	<0.001
Gram (–ve)	1.9	1.4	1.4	0.34	0.4541
Gram (+ve)	3.7	2.8	1.6	0.74	0.1841

^{abc}Means with different superscripts in a row differ (**p* < 0.05, ****p* < 0.001)

[‡]BSP black walnut silvopasture, HSP honeylocust silvopasture, OP open pasture, SE standard error of means

[†]PLFA phospholipid fatty acid, AMF arbuscular mycorrhizal fungi

Discussion

Greater soil OM content in HSP relative to OP was observed in this study. This can result in improved soil structure, nutrient availability, and overall soil health in the system (Johnston et al., 2009). Greater soil OM content in savannas and silvopastures compared to open pastures and grasslands has been reported in several studies (Casals et al. 2014; Belsky et al. 1993; Rhoades 1997). Several mechanisms may have played a role in improved soil OM in HSP compared to OP. Increased soil OM under HSP could reflect greater above- and below-ground litter inputs or lower decomposition rates compared to the open pasture treatment and may also be a function of the floristic composition of the herbaceous vegetation. Although not measured for this study, aboveground primary productivity in HSP has been comparable to production in OP systems (Fannon et al. 2017; Pent et al. 2020). The compositional difference in available vegetation in HSP and OP systems has also been reported by a previous study at this research site (Pent et al. 2020). Greater relative biomass yield and differences in vegetation composition coupled with lower temperatures within the silvopasture may have helped drive the greater soil OM in these systems. Factors

supporting higher soil OM content in HSP compared to BSP might include differences in root architecture between tree species (Akinnesi et al. 1999). Black walnut trees form a taproot while honeylocust trees do not and greater surface roots are common in non-taproot-forming trees (Akinnesi et al. (1999). Leaf morphology and physiochemical properties may also affect this response as they influence the potential decomposition rate of leaf litter (Cornelissen 1996). Higher soil OM content in a system can increase soil organic C thereby helping in the restoration of better soil health and fertility.

Soil bulk density and soil pH did not differ by treatment in this study. In contrast, Tonucci et al. (2011) reported a significantly greater soil bulk density and soil pH (0–10 cm) in open pasture compared to eucalyptus- (*Eucalyptus sp.*) based silvopasture in a tropical climate. This contrasting finding among these studies might be due to the differences in soils between the tropical system in the study by Tonucci et al. (2011) versus the temperate system in the current study. The insignificant difference in soil bulk density and soil pH among treatments in this study may reflect similarities in management across systems from the time of its establishment. In this study, both silvopasture and open pasture treatments were established within an existing cool-season pasture and have received similar fertility and grazing management over time. These management inputs may have buffered the system, limiting the opportunity to observe differences driven by the presence of the deciduous hardwood trees. Land-use history and management practices play a significant role in altering soil bulk density (Kahlon and Khurana 2017).

Soil P concentrations did not differ among treatments in this study. A study by Nair et al. (2007) reported a similar Mehlich-1 P concentration among treeless pasture with bahiagrass (*Paspalum notatum*) and 20-year-old slash pine (*Pinus elliotti*) silvopasture at 0–5 cm depth. This insignificant difference in P concentration among treatments might be due to limited soil sampling depth. In this study, soil samples were collected from 0 to 10 cm depth, but trees remove P from deeper depths within the soil profile where differences would be much more prominent due to the presence of trees. Greater K⁺ in HSP soils compared to OP soils is likely due to higher soil OM and enhanced nutrient extraction and cycling by the trees' root systems (Buresh and Tian 1998). Similarly,

the trend of greater Ca^{2+} concentration in HSP soils compared to OP soils may help explain the lower soil acidity in HSP compared to the OP treatment. Also, litterfall in BSP and HSP could represent a major influx of Ca^{2+} to soils (Campo et al. 2001). Various studies have reported similar findings of elevated cations in soils under the trees (Alfaia et al. 2004; Casals et al. 2014).

Soil total C and MBC content were higher in silvopasture treatments compared to OP and these differences might be due to differences in above- and belowground litter inputs or lower decomposition rates in silvopasture treatments compared to OP. Root biomass is usually considered a key source of C inputs to the soil and is converted to soil organic C by microbial activity; although root biomass is assumed to be greater in silvopasture treatments, it was not measured in this study. Ramakrishnan et al. (2020) reported 15–61% greater MBC in a bael (*Aegle marmelos*) silvopasture system compared to monoculture pasture consisting of buffelgrass (*Cenchrus ciliaris*), Caribbean stylo (*Stylosanthes hamata*), and Caatinga stylo (*Stylosanthes seabrana*). Similarly, Haile et al. (2008) reported 28–33% greater total soil organic C content in slash pine (*Pinus elliottii*) and bahiagrass- (*Paspalum notatum*) based silvopastures than in bahiagrass pastures. Silvopastures that increase microbial diversity and complexity have the potential to sequester more C than forests or a monoculture pasture system (Sharrow and Ismail 2004). However, the age of the system, management practices, climate, soil conditions, and land-use history can play a significant role in the amount of C accumulated in an ecosystem (Peichl et al. 2006). Silvopasture soils had greater MBN along with a strong correlation of MBN with $\text{NO}_3\text{-N}$ ($r=0.99$). The microbial biomass C in the soil is highly influenced by soil OM content and this difference in MBC between silvopasture and OP treatments might reflect greater soil OM and enhanced nutrient extraction and cycling by the trees' root systems in silvopasture compared to OP (Buresh and Tian 1998). Thus higher soil OM or above- and below-ground litter inputs in silvopasture increased MBC in the soil thereby helping in restoring better soil health.

A trend toward greater GRSP contents in HSP compared to OP might be due to the amount and quality of above- and below-ground litter inputs along with no significant difference in the occurrence

of AMF density among the treatments. The amount of plant residue inputs and the degree of soil OM decomposition plays a key role in soil GRSP levels. Soil enzymes play a key role in maintaining nutrient cycling and are very responsive to various natural and anthropogenic factors. Therefore, soil enzymatic activities can be considered a sensitive indicator to evaluate soil health. Soil β -glucosidase activity is often related to the amount and characteristics of organic C inputs; land management practices that promote accumulation of C and N are expected to increase β -glucosidase activity in soil as well (Weintraub et al. 2013). Greater β -glucosidase activity in silvopasture treatments compared to open pasture likely reflect differences in C inputs and degradability associated with tree root and litterfall contributions. Similarly, urease activity was higher in BSP compared to OP treatment. Urease activity in soil is highly dependent on organic C and total N indicating higher OM content in the soil which is greater in BSP compared to OP. Zantua et al. (1977) suggested that the variation in urease activity in soil is predominantly dependent on the OM content of the soil.

The composition and abundance of microbial communities in the soil are critical for the maintenance of soil ecosystem services and overall soil health. These microbes degrade plant-derived C to produce microbial biomass that is later stabilized as necromass through mineral stabilization and in soil aggregates (Liang 2020). A cluster in soil microbial community structure was observed within different treatments, suggesting the systems provide unique ecological niches for soil microbial communities in response to the C inputs within the system (Hopkins et al. 2014). Silvopasture treatments had greater bacterial abundance and lower abundance of AMF than open pasture. An increase in bacterial and decrease in AMF abundance in silvopasture treatments compared to open pasture might be due to a difference in system composition that ultimately altered the organic matter composition and substrate for microorganisms thus altering the microbial community composition (Marschner et al. 2003). Total PLFA and actinomycetes abundance were greater in OP soils compared to BSP soils. These data stand in contrast with various studies from tropical and subtropical regions in which greater microbial community abundance was observed in silvopasture relative to monoculture pastures (Vallejo et al. 2012; Cubillos et al. 2016; Ramakrishnan et al.

2020). Lower PLFA and actinomycetes abundance in BSP compared to OP might be the effect of juglone, an allelochemical produced by black walnut trees that could be detrimental to the soil microbial community. However, while juglone has a modest repressive effect on GP and acid-fast bacteria as well as has moderate antifungal activity (Clark et al. 1990), no difference in GP and GN bacterial abundance was observed among the treatments. Bacterial abundance was greater than fungal abundance for the soils in all systems. Within the bacterial fraction, GP bacteria were more abundant than GN bacteria, indicating GP dominated the microbial biomass. The ratio of fungal to bacterial abundance was higher in OP (0.16) compared to silvopasture treatments (0.10). Higher fungal abundance is associated with the improved capacity of soil to sequester C thus enhancing organic C accumulation and decreased in its turnover rate (Malik et al. 2016). However, no difference in fungal abundance between treatments was observed in this study. The ratio of fungal and bacterial abundance in the soil is influenced by C:N ratio, with a higher C:N ratio favoring higher fungal abundance (Lundquist et al. 1999). In this study, the C:N ratio was relatively low for all soils, ranging from 10.2 to 10.7, and lower fungal abundance likely reflects a low C:N ratio that favors bacteria (Fierer et al. 2009).

Conclusions

This study indicates that physiochemical properties, nutrient dynamics, and overall soil health were moderately improved in mid-stage (25-years-old) temperate hardwood silvopastures compared to treeless pastures. Soil OM, total C, GRSP, MBC, and MBN at 0–10 cm depth were greater in silvopasture treatments than OP. Acid phosphatase, β -glucosidase, and urease activities were also increased in silvopasture soil compared to OP soil. The total PLFA, actinomycetes, and AMF abundance were greater in OP compared to BSP while total bacterial, total fungal, GP, and GN bacterial abundance did not differ among treatments. Such changes within silvopasture systems greatly depend upon tree characteristics, age of the system, and its management. Trees and understory vegetation within the silvopastures can help improve C inputs to the soil and are practically applicable in C accretion and agricultural sustainability in the long run. Further

study is needed to determine the effect of trees on soil physiochemical properties and nutrient dynamics at greater depth and as this silvopasture system matures.

Acknowledgements The authors would like to acknowledge Virginia Tech Soil Testing Lab, Virginia Tech Environmental Quality Lab, and Soil Physics Lab at South Dakota State University for help with all the lab analysis.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

- Akinnifesi FK, Kang BT, Ladipo DO (1999) Structural root form and fine root distribution of some woody species evaluated for agroforestry systems. *Agrofor Syst* 42(2):121–138. <https://doi.org/10.1023/A:1006178002019>
- Alfaia SS, Ribeiro GA, Nobre AD, Luizao RC, Luizao FJ (2004) Evaluation of soil fertility in smallholder agroforestry system and pastures in western Amazonia. *Agric Ecosyst Environ* 102:409–414. <https://doi.org/10.1016/j.agee.2003.08.011>
- Beck T, Joergensen RG, Kandeler E, Makeschin F, Nuss E, Oberholzer HR, Scheu S (1997) An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biol Biochem* 29(7):1023–1032. [https://doi.org/10.1016/S0038-0717\(97\)00030-8](https://doi.org/10.1016/S0038-0717(97)00030-8)
- Belsky AJ, Mwonga SM, Duxbury JM (1993) Effects of widely spaced trees and livestock grazing on understory environments in tropical savannas. *Agrofor Syst* 24:1–20
- Bendfeldt ES, Feldhake CM, Burger JA (2001) Establishing trees in an Appalachian silvopasture: response to shelters, grass control, mulch, and fertilization. *Agrofor Syst* 53:291–295. <https://doi.org/10.1023/A:1013367224860>
- Bosi C, Pezzopane JRM, Sentelhas PC (2020) Soil water availability in a full sun pasture and in a silvopastoral system with eucalyptus. *Agrofor Syst* 94:429–440. <https://doi.org/10.1007/s10457-019-00402-7>
- Buresh RJ, Tian G (1998) Soil improvement in sub-Saharan Africa. *Agrofor Syst* 38:51–76. https://doi.org/10.1007/978-94-015-9008-2_2
- Campo J, Maass M, Jaramillo VJ, Martínez-Yrizar A, Sarukhán J (2001) Phosphorus cycling in a Mexican tropical dry forest ecosystem. *Biogeochemistry* 53(2):161–179. <https://doi.org/10.1023/A:1010663516029>
- Casals P, Romero J, Rusch GM, Ibrahim M (2014) Soil organic C and nutrient contents under trees with different functional characteristics in seasonally dry tropical silvopastures. *Plant Soil* 374(1):643–659. <https://doi.org/10.1007/s11104-013-1884-9>
- Clapperton M, Lacey M, Hanson K, Hamel C (2005) Analysis of phospholipid and neutral lipid fatty acids extracted from soils. *Research Newsletter*. Swift Current, SK: SPARC-AAFC December 12

- Clark AM, Jurgens TM, Hufford CD (1990) Antimicrobial activity of juglone. *Phytopath Res* 4(1):11–14. <https://doi.org/10.1002/ptr.2650040104>
- Cornelissen JHC (1996) An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *J Ecol*. <https://doi.org/10.2307/2261479>
- Cubillos AM, Vallejo VE, Arbeli Z, Teran W, Dick RP, Molina CH, Molina E, Roldan F (2016) Effect of the conversion of conventional pasture to intensive silvopastoral systems on edaphic bacterial and ammonia oxidizer communities in Colombia. *Eur J Soil Biol* 72:42–50. <https://doi.org/10.1016/j.ejsobi.2015.12.003>
- Eivazi F, Tabatabai MA (1977) Phosphatases in soils. *Soil Biol Biochem* 9(3):167–172
- Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. *Soil Biol Biochem* 20(5):601–606
- Fannon AG, Fike JH, Greiner SP, Feldhake CM, Wahlberg MA (2017) Hair sheep performance in a mid-stage deciduous Appalachian silvopasture. *Agrofor Syst*. <https://doi.org/10.1007/s10457-017-0154-x>
- Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. *Ecol Lett* 12:1238–1249. <https://doi.org/10.1111/j.1461-0248.2009.01360.x>
- Garrett HE, Rietveld WJ, Fisher RF (2000) North American agroforestry: an integrated science and practice. American Society of Agronomy, Madison
- Ghani A, Dexter M, Perrott KW (2003) Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biol Biochem* 35(9):1231–1243. [https://doi.org/10.1016/S0038-0717\(03\)00186-X](https://doi.org/10.1016/S0038-0717(03)00186-X)
- Gregorich EG, Wen G, Voroney RP, Kachanoski RG (1990) Calibration of a rapid direct chloroform extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 22(7):1009–1011. [https://doi.org/10.1016/0038-0717\(90\)90148-S](https://doi.org/10.1016/0038-0717(90)90148-S)
- Haile SG, Nair PR, Nair VD (2008) Carbon storage of different soil-size fractions in Florida silvopastoral systems. *J Environ Qual* 37(5):1789–1797. <https://doi.org/10.2134/jeq2007.0509>
- Hawken P (2017) Drawdown: the most comprehensive plan ever proposed to reverse global warming. Penguin, London
- Hopkins MF, Filley RT, Gleixner G, Lange M, Top MS, Trumbore ES (2014) Increased belowground carbon inputs and warming promote loss of soil organic carbon through complementary microbial responses. *Soil Biol Biochem* 76:57–69. <https://doi.org/10.1016/j.soilbio.2014.04.028>
- Jackson RB, Schenk HJ, Jobbagy EG, Canadell J, Colello GD, Dickinson RE, Field CB, Friedlingstein P, Heimann M, Hibbard K, Kicklighter DW (2000) Belowground consequences of vegetation change and their treatment in models. *Ecol Appl* 10(2):470–483. [https://doi.org/10.1890/1051-0761\(2000\)010\[0470:BCOVCA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[0470:BCOVCA]2.0.CO;2)
- Janzen HH, Janzen DW, Gregorich EG (2021) The ‘soil health’ metaphor: Illuminating or illusory? *Soil Biol Biochem* 159:108167. <https://doi.org/10.1016/j.soilbio.2021.108167>
- Johnston AE, Poulton PR, Coleman K (2009) Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. *Adv Agron* 101:1–57. [https://doi.org/10.1016/S0065-2113\(08\)00801-8](https://doi.org/10.1016/S0065-2113(08)00801-8)
- Kahlon MS, Khurana K (2017) Effect of land management practices on physical properties of soil and water productivity in the wheat-maize system of Northwest India. *Appl Ecol Environ Res* 15(4):1–13. https://doi.org/10.15666/aeer/1504_001013
- Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol Fertil Soils* 6(1):68–72. <https://doi.org/10.1007/BF00257924>
- Karki S, Shange R, Ankumah R, McElhenney W, Idehen O, Poudel S, Karki U (2021) Comparative assessment of soil health indicators in response to woodland and silvopasture land use systems. *Agrofor Syst* 95(1):227–240. <https://doi.org/10.1007/s10457-020-00577-4>
- Liang C (2020) Soil microbial carbon pump: mechanism and appraisal. *Soil Ecol Lett* 2(4):241–254. <https://doi.org/10.1007/s42832-020-0052-4>
- Lundquist EJ, Jackson LE, Scow KM, Hsu C (1999) Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. *Soil Biol Biochem* 31:221–236. [https://doi.org/10.1016/S0038-0717\(98\)00093-5](https://doi.org/10.1016/S0038-0717(98)00093-5)
- Malik AA, Chowdhury S, Schlager V, Oliver A, Puissant J, Vazquez PG, Jehmlich N, Von Bergen M, Griffiths RI, Gleixner G (2016) Soil fungal: bacterial ratios are linked to altered carbon cycling. *Front Microbiol* 7:1247. <https://doi.org/10.3389/fmicb.2016.01247>
- Marschner P, Kandeler E, Marschner B (2003) Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biol Biochem* 35(3):453–461
- Nair VD, Nair PKR, Kalmbacher RS, Ezenwa IV (2007) Reducing nutrient loss from farms through silvopastoral practices in coarse-textured soils of Florida, USA. *Ecol Eng* 29(2):192–199. <https://doi.org/10.1016/j.ecoleng.2006.07.003>
- Nelson DW, Sommers LE (1996) Total carbon, organic carbon and organic matter. In: Sparks DL (ed) *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Ser. 5. SSSA, Madison, pp 961–1010
- Peichl M, Thevathasan NV, Gordon A, Huss J, Abohassan RA (2006) Carbon sequestration potentials in temperature tree-based intercropping systems, southern Ontario, Canada. *Agrofor Syst* 66:243–257
- Pent GJ (2020) Over-yielding in temperate silvopastures: a meta-analysis. *Agrofor Syst* 94(5):1741–1757. <https://doi.org/10.1007/s10457-020-00494-6>
- Pent GJ, Greiner SP, Munsell JF, Tracy BF, Fike JH (2020) Lamb performance in hardwood silvopastures, I: animal gains and forage measures in summer. *Transl Anim Sci* 4(1):385–399. <https://doi.org/10.1093/tas/txz154>
- Ramakrishnan S, Kumar S, Chaudhary M, Govindasamy P, Yadav M, Prasad M, Srivastava R, Kumari B, Prajapati K (2020) Silvopastoral system for resilience of key soil health indicators in semi-arid environment. *Arch Agron Soil Sci*. <https://doi.org/10.1080/03650340.2020.1814954>
- Rhoades CC (1997) Single-tree influences on soil properties in agro-forestry: lessons from natural forest and savanna

- ecosystems. *Agrofor Syst* 35:71–94. <https://doi.org/10.1007/BF02345330>
- Schroth G (1995) Tree root characteristics as criteria for species selection and systems design in agroforestry. *Agrofor Syst* 30:125–143. https://doi.org/10.1007/978-94-017-0681-0_6
- Sharrow SH, Ismail S (2004) Carbon and nitrogen storage in agroforests, tree plantations, and pastures in western Oregon, USA. *Agrofor Syst* 60(2):123–130. <https://doi.org/10.1023/B:AGFO.0000013267.87896.41>
- Staley TE, Gonzalez JM, Neel JP (2008) Conversion of deciduous forest to silvopasture produces soil properties indicative of rapid transition to improved pasture. *Agrofor Syst* 74(3):267. <https://doi.org/10.1007/s10457-008-9139-0>
- Tabatabai MA, Bremner JM (1972) Assay of urease activity in soils. *Soil Biol Biochem* 4(4):479–487. [https://doi.org/10.1016/0038-0717\(72\)90064-8](https://doi.org/10.1016/0038-0717(72)90064-8)
- Tonucci RG, Nair PR, Nair VD, Garcia R, Bernardino FS (2011) Soil carbon storage in silvopasture and related land-use systems in the Brazilian Cerrado. *J Environ Qual* 40(3):833–841. <https://doi.org/10.2134/jeq2010.0162>
- USDA NRCS (Natural Resources Conservation Service) (2014). Soil health home. <https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/>. Accessed 23 April 2022
- Vallejo VE, Arbeli Z, Terán W, Lorenz N, Dick RP, Roldan F (2012) Effect of land management and *Prosopis juliflora* (Sw.) DC trees on soil microbial community and enzymatic activities in intensive silvopastoral systems of Colombia. *Agric Ecosyst Environ* 150:139–148. <https://doi.org/10.1016/j.agee.2012.01.022>
- Watson ME, Brown JR (1998) pH and lime requirement. Recommended chemical soil test procedures for the North Central Region. North Central Regional Research Publication No. 221 (Revised). Missouri Agricultural Experiment Station SB 1001, Columbia
- Weintraub SR, Wieder WR, Cleveland CC, Townsend AR (2013) Organic matter inputs shift soil enzyme activity and allocation patterns in a wet tropical forest. *Biogeochemistry* 114(1):313–326. <https://doi.org/10.1007/s10533-012-9812-2>
- Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* 198(1):97–107. <https://doi.org/10.1023/A:1004347701584>
- Zantua MI, Dumenil LC, Bremner JM (1977) Relationships between soil urease activity and other soil properties. *Soil Sci Soc Am J* 41(2):350–352. <https://doi.org/10.2136/sssaj1977.03615995004100020036x>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.