

## ARTICLE

## Soil Fertility and Crop Nutrition

# Improvements in soil properties under adaptive multipaddock grazing relative to conventional grazing

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**Abstract**

Within managed ecosystems, such as some livestock grazed grasslands, soil physical, chemical, and biological properties may be severely compromised relative to native grasslands. Conventional grazing (CG) management, commonly referred to as continuous grazing, can affect soil properties and health by reducing soil C stocks and other available nutrients, while creating bare patches in vegetation that may enhance erosion and runoff. In contrast, adaptive multipaddock (AMP) grazing, an intensive form of rotational grazing that moves dense cattle herds quickly over the land followed by rest periods for the regrowth of plants, has been proposed as a regenerative grassland management tool that can improve soil properties such as soil C stocks, soil structure, as well as nutrient and water retention. Our research analyzed soils from 10 grasslands in the southeast United States representing either CG or AMP grazing management. We analyzed the A-horizons of these soils for physical, chemical, and biological properties considered indicators of soil health across each management type. Chemical soil properties (e.g., cation exchange capacity [CEC], base saturation [BS], electrical conductivity [EC]) were improved where AMP grazing management was implemented. Additionally, farms using AMP grazing management had greater A-horizon C and N stocks in bulk soils and across multiple soil organic matter (SOM) fractions. No biological indicators measured were affected by the grassland management except potential N mineralization rate, which was lower under AMP. Taken together, these results provide evidence that AMP grazing management could be implemented to regenerate several grassland soil properties across land currently under conventional grazing management.

**Abbreviations:** AMP, adaptive multipaddock; BS, base saturation; CEC, cation exchange capacity; CG, conventional grazing; DOM, dissolved organic matter; EC, electrical conductivity; HPOM, heavy particulate organic matter; LPOM, light particulate organic matter; MAOM, mineral-associated organic matter; MWD, mean weight diameter; PLFA, phospholipid fatty acid; SOM, soil organic matter; WHC, water holding capacity.

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## 1 | INTRODUCTION

Broadly, soil health refers to soils that are able to support food and fiber production while also providing and maintaining ecosystem services (Doran & Parkin, 1994; Kibblewhite et al., 2008; Larson & Pierce, 1991). Soil health metrics encompass soil physical, chemical, and biological properties, as they are all important for regulating a healthy soil environment. Improving these soil properties can lead to increasing plant productivity, water quality, ecosystem resilience to drought and other extreme weather events, soil C sequestration, and reductions in greenhouse gas emissions (Byrnes et al., 2018; Kibblewhite et al., 2008). Thus, soil health has attracted a lot of attention, and several soil health initiatives have been developed to further research soil properties indicative of healthy soils (Andrews et al., 2004; Jian et al., 2020; Moebius-Clune et al., 2016; Norris et al., 2020).

Many organizations promote sustainable soils by improving soil health (USDA, NRCS, Soil Health Institute, etc.). For example, the Soil Health Institute created two lists of soil properties to measure when monitoring for soil health (Norris et al., 2020). The Tier 1 list consists of soil properties that are well documented as relating to crop yields and have been responsive to management improvements (e.g., soil pH, electrical conductivity [EC], cation exchange capacity [CEC], percentage base saturation [BS], extractable nutrients, texture, C and N concentrations, water holding capacity [WHC], aggregation, and C and N mineralization potentials), whereas Tier 2 consists of properties that are less understood in terms of improving soil health (e.g., extracellular enzyme activities, phospholipid fatty acids [PLFAs], genomics, reflectance) (Norris et al., 2020). Organizations promoting measurements of these soil properties, the Soil Health Institute included, tend to include a large proportion of chemical properties, which highlights the importance of these indicators for maintaining a healthy soil (Lehmann et al., 2020). However, soil organic matter (SOM) fractionation is excluded from many soil health analyses including the Soil Health Institute (Norris et al., 2020). The SOM fractions are often more sensitive to carbon stock changes than bulk soil C measurements, and they can reveal more information about soil C such as mechanisms and pathways of formation as well as persistence and vulnerability to changes (Cotrufo et al., 2015; Lavallee et al., 2020; Rocci et al., 2021). Therefore, we posit that soil health tests can benefit from the inclusion of SOM fractions in addition to bulk soil C measurements. This holistic approach to quantify physical, chemical, and biological properties of soil health may be useful for comparison between management practices especially when current soils may be improved using best management practices.

One such example of potential for improving soil health in grazing land can be found within the context of conventional,

### Core Ideas

- We found improved chemical soil health indicators where AMP grazing was implemented.
- Greater AMP A-horizon C and N stocks were apparent across multiple SOM fractions.
- Biological soil health indicators were largely unaffected by grazing management.
- Results show that AMP grazing could be used to regenerate grassland soil health.

or continuous, grazing (CG). Overgrazing can be common in CG systems, which can negatively affect soil health by reducing soil C (Conant et al., 2001; Teague, 2018), increasing compaction and bulk density (Bailey et al., 1998; Steffens et al., 2008; Teague et al., 2004; Teague et al., 2016), and degrading vegetation that leads to reduced plant productivity, bare soil, erosion, and runoff of essential nutrients (Milchunas & Laurenroth, 1993). Adaptive multipaddock (AMP) grazing management is an intensive form of rotational grazing that moves dense cattle herds quickly over the land followed by adequate rest periods for the regrowth of plants. By having the cattle in greater concentrations, inputs of feces and urine are more evenly distributed across the soil (Teague, 2018). Cattle are quickly moved from paddock to paddock (typically every 1–4 d) based on available forage resources (Shewmaker & Bohle, 2010), allowing soil to rest from grazing disturbance and vegetation to regrow after the grazing episode. Previous research has shown that rotational grazing can increase soil C (Conant & Paustian, 2002; Machmuller et al., 2014; Teague et al., 2011), improve bulk density (Byrnes et al., 2018; Teague et al., 2013) and soil structure (Teague, 2018), increase nutrient and water retention (Franzluebbers & Stuedemann, 2009; Shawver et al., 2020), and reduce erosion (Teague et al., 2013), all which may lead to improved soil health.

However, the above studies have only looked at a few soil properties, creating a clear need for a full soil health assessment quantifying changes in physical, chemical, and biological soil property between AMP and CG management. Thus, we performed, to our knowledge, the first regional-scale study analyzing many Soil Health Institute Tier 1 and Tier 2 soil properties, with the addition of SOM fractions quantification, and compared them between AMP and CG farms (Table 1). Specifically, this study analyzed soils from five paired ‘across-the-fence’ grazed grasslands in the southeast region of the United States, representing either AMP or CG management, supporting the direct comparison of contrasting management strategies on the same soil type and slopes with similar aspects (Mosier et al., 2021).

**TABLE 1** List of soil health indicators measured for this study separated by physical, chemical, and biological metrics. Each indicator unit is reported as well as the common acronym used for reference in text, tables, and figures

Indicator	Acronym	Units	Average values $\pm$ SE	
			AMP	CG
<b>Physical subgroup</b>				
A-horizon depth	Adepth	cm	12.61 $\pm$ 0.27	11.75 $\pm$ 0.21
Sand/clay ratio	Sand.Clay	–	1.82 $\pm$ 0.29	<b>2.26 <math>\pm</math> 0.28</b>
Mean-weight diameter	MWD	mm	2.26 $\pm$ 0.13	3.26 $\pm$ 0.13
Available water holding capacity	WHC	cm water cm soil <sup>-1</sup>	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01
Dissolved organic matter	DOM	Mg C ha <sup>-1</sup>	<b>1.04 <math>\pm</math> 0.06</b>	0.88 $\pm$ 0.05
Light particulate organic matter	LPOM	Mg C ha <sup>-1</sup>	6.19 $\pm$ 0.27	5.61 $\pm$ 0.35
Heavy particulate organic matter	HPOM	Mg C ha <sup>-1</sup>	7.27 $\pm$ 0.35	6.25 $\pm$ 0.25
Mineral-associated organic matter	MAOM	Mg C ha <sup>-1</sup>	<b>19.86 <math>\pm</math> 0.73</b>	17.02 $\pm$ 0.64
<b>Chemical subgroup</b>				
Organic carbon stocks	C.stock	Mg C ha <sup>-1</sup>	<b>31.51 <math>\pm</math> 0.60</b>	26.99 $\pm$ 0.46
Nitrogen stocks	N.stock	Mg N ha <sup>-1</sup>	<b>3.40 <math>\pm</math> 0.06</b>	2.82 $\pm$ 0.05
pH	pH	–	<b>5.90 <math>\pm</math> 0.11</b>	5.60 $\pm$ 0.06
Base saturation	BS	%	<b>56.32 <math>\pm</math> 2.39</b>	48.34 $\pm$ 1.39
Electrical conductivity	EC	dS m <sup>-1</sup>	<b>0.23 <math>\pm</math> 0.01</b>	0.15 $\pm$ 0.01
Cation exchange capacity	CEC	cmolc kg soil <sup>-1</sup>	<b>15.62 <math>\pm</math> 0.89</b>	11.72 $\pm$ 0.91
Calcium	Ca	mg kg soil <sup>-1</sup>	<b>1357.73 <math>\pm</math> 114.96</b>	831.01 $\pm$ 67.13
Magnesium	Mg	mg kg soil <sup>-1</sup>	<b>143.85 <math>\pm</math> 11.22</b>	116.10 $\pm$ 9.01
Potassium	K	mg kg soil <sup>-1</sup>	<b>238.52 <math>\pm</math> 18.41</b>	148.82 $\pm$ 6.87
Sodium	Na	mg kg soil <sup>-1</sup>	3.59 $\pm$ 1.03	4.12 $\pm$ 1.26
Zinc	Zn	mg kg soil <sup>-1</sup>	3.83 $\pm$ 0.34	3.45 $\pm$ 0.48
Manganese	Mn	mg kg soil <sup>-1</sup>	182.02 $\pm$ 24.21	167.37 $\pm$ 19.13
Sulfur	S	mg kg soil <sup>-1</sup>	12.04 $\pm$ 0.25	11.55 $\pm$ 0.37
Copper	Cu	mg kg soil <sup>-1</sup>	2.96 $\pm$ 0.18	4.10 $\pm$ 0.46
Iron	Fe	mg kg soil <sup>-1</sup>	204.28 $\pm$ 21.31	204.41 $\pm$ 12.19
Ammonium concentration	NH4	mg L <sup>-1</sup>	1.93 $\pm$ 0.34	<b>3.00 <math>\pm</math> 0.23</b>
Nitrate concentration	NO3	mg L <sup>-1</sup>	<b>5.13 <math>\pm</math> 0.27</b>	3.97 $\pm$ 0.49
<b>Biological subgroup</b>				
Carbon mineralization	Cmin	mg C g C <sup>-1</sup>	100.71 $\pm$ 3.51	106.50 $\pm$ 6.99
Nitrogen mineralization	Nmin	mg N g N <sup>-1</sup>	4.53 $\pm$ 0.59	<b>7.30 <math>\pm</math> 0.98</b>
Carbon enzymes	Cenz	nmol activity g soil <sup>-1</sup>	134.78 $\pm$ 9.98	169.75 $\pm$ 23.91
Nitrogen enzymes	NAG	nmol activity g soil <sup>-1</sup>	59.20 $\pm$ 4.07	78.46 $\pm$ 14.86
Phosphorus enzymes	PHOS	nmol activity g soil <sup>-1</sup>	195.92 $\pm$ 17.37	244.80 $\pm$ 25.16
Total phospholipid fatty acids	PLFA	$\mu$ g soil <sup>-1</sup>	5.87 $\pm$ 0.33	5.76 $\pm$ 0.34
Fungi/bacteria phospholipid fatty acids	Fungi.Bact	–	0.09 $\pm$ 0.004	0.09 $\pm$ 0.005

Note. Also reported are the adaptive multipaddock grazing (AMP) and conventional grazing (CG) farm average values  $\pm$  SE for each soil health indicator measured. Significantly higher mean differences are bolded.

By analyzing the suite of soil physical, chemical, and biological properties recommended by the Soil Health Institute, we were able to better understand (a) how grazing practices affect soil health and (b) which soil properties explain best, for monitoring purposes, the differences generated by grazing management.

## 2 | METHODS

### 2.1 | Study sites

The study sites used for this study were the same used in Mosier et al (2021), where we provide their detailed

description. Briefly, they were located across four states (Kentucky, Tennessee, Alabama, and Mississippi) and included five sets of paired AMP grazing and CG farms practicing their current management regime for at least 10 years. Our study sites were identified through a careful process of understanding biophysical conditions and land management history and screening by ecologists, soil scientists, and grazing experts to identify which AMP managed farms and paired CG farms to include in the study (Mosier et al., 2021). Through this process, we confirmed that each AMP farm and their paired CG neighboring farm were located on the same soil type, yet the farm pairs represented a wide range of soil types from the southeast U.S. (Mosier et al., 2021). For the purpose of the study, AMP grazing management farms were defined by having >40 paddocks, stocking rates >1 animal unit ha<sup>-1</sup>, stocking densities >60 animal unit ha<sup>-1</sup>, and a rest/grazed ratio of >40 d. All CG management farms had values below the above thresholds and represented the most common grazing practices in this region (Mosier et al., 2021).

## 2.2 | Soil sampling and processing

At each grazed farm, we sampled two catenas, each across three different transect zones—upper, middle, and lower slopes—to account for landscape topography and heterogeneity but representing the same soil type, slope, and aspect across each farm pair. Sampling points were randomly placed in each transect zone. At each farm, we sampled seven 1-m deep, 4-cm diam. cores per transect zone using a hydraulic Giddings unit for a total of 42 cores per farm and 420 soil cores total. More soil sampling details can be found in Mosier et al. (2021). Soils were then shipped intact to Colorado State University where the A-horizon depth was separated from the rest of the core. We recorded the A-horizon depth and then used the A-horizon as a metric of soil health and regeneration. For this study, we focused on the A-horizon samples because this layer is the active soil horizon, the richest in organic matter, and where the majority of plant and soil organism activity occurs (Stott & Moebius-Clune, 2017).

After the horizon separation, the A-horizons were passed through an 8-mm sieve to remove rocks, roots, and litter. We then composited a portion of the A-horizon samples by transect to create one representative sample for each transect; there were six transects per farm, resulting in a total of 60 composited A-horizon samples to analyze for soil properties. A portion of the composited A-horizons was placed in a -80 °C freezer as fresh soil for microbial analyses, inorganic N concentrations, and N mineralization potentials. Another subset of the composited A-horizons was air dried for extractable nutrients, C mineralization potentials, aggregation, mean weight diameter (MWD), pH, EC, BS, and CEC. The remaining soil was 2-mm-sieved and dequarantined by

heat treatment in a 110 °C oven to determine gravimetric moisture content, texture, and SOM fractions. We also kept a subsample of the 8-mm-sieved individual core A-horizon soils ( $n = 420$ ), which we then 2-mm sieved (removing rocks, roots, and litter) and dequarantined by heat treatment to analyze total soil organic C and total soil N. The mass of the removed materials was used to correct the soil core volume for bulk density (Mosier et al., 2019). The A-horizon SOM fraction data as well as the total soil organic C and total soil N stocks data are a subset of the 1-m-deep soil stocks reported in Mosier et al. (2021).

## 2.3 | Physical soil properties

We used the hydrometer method to determine soil texture (Gee & Bauder, 1986). Briefly, we shook 40 g of 2-mm-sieved oven-dried soil for 18 h with 100 ml of sodium hexametaphosphate (50 g L<sup>-1</sup>) to break up aggregates. We then added the soil slurry to a gravimetric flask, and density was sampled at time 0, 40 s, and at 2 h. We measured water-stable aggregates using a wet sieving procedure that determines aggregate stability of four aggregate sizes: >2 mm, 2 mm–250 μm, 250–53 μm, and <53 μm (Kemper & Roseneau, 1986). All aggregate size classes were corrected for rock and sand particles. After correction, we estimated MWD by multiplying the proportion of each aggregate size class by the median diameter of each size class and then summing them together to get one value (Kemper & Roseneau, 1986). Additionally, we measured available WHC on ground soil samples using the ceramic plate method measured at one-third and 15 bars (Klute, 1986), with available WHC measured as the difference between the two tensions.

We physically separated SOM by size and density into four different fractions: dissolved organic matter (DOM; readily bioavailable), light particulate organic matter (LPOM; mostly plant and microbial structural compounds in the early stages of decomposition), heavy particulate organic matter (HPOM; more decomposed plant and microbial compounds coating larger particles or within highly stable aggregates), and mineral-associated organic matter (MAOM; mostly plant soluble and microbial compounds chemically bonded to minerals) following Mosier et al. (2021). To obtain the DOM fraction, we shook 30 ml deionized H<sub>2</sub>O with a 10 g subsample of 2-mm sieved oven-dried soil, centrifuged the sample, and then collected the DOM fraction. We added sodium polytungstate (1.85 g cm<sup>-3</sup>) to the remaining soil and shook the sample for 18 h to break up aggregates. We then centrifuged the sample, which separated the SOM by density, allowing us to aspirate off the LPOM fraction. We rinsed the soil of any residual sodium polytungstate and then size separated the remaining soil by wet sieving into HPOM (>53 μm) and MAOM (<53 μm).

## 2.4 | Chemical soil properties

We analyzed all the SOM fractions mentioned above (excluding DOM) as well as the bulk soil for percentage C and percentage N using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies). The DOM fraction was analyzed for total organic C and total N using a Shimadzu TOC-L/TNM-L Analyzer (Shimadzu Corporation). Additionally, we tested our soils for inorganic C with an acid pressure transducer and a voltage meter (Sherrod et al., 2002) and, where present, we removed any measured inorganic C from the total C to obtain total organic C. Bulk soil organic C and N stocks, as well as each SOM fraction C stock, was determined using percentage C and percentage N concentrations and bulk density measurements as reported in Mosier et al. (2021).

We determined pH and EC using a 1:1 soil to deionized H<sub>2</sub>O slurry and a pH electrode (Rhoades, 1996; Thomas, 1996). Briefly, we placed 20 g of soil and 20 ml of deionized H<sub>2</sub>O in a 50-ml centrifuge tube, shook for 2 h and then measured pH directly in the slurry. Tubes were then centrifuged to separate the solution phase, with the solution gently poured into an electrical conductivity meter for EC determination. Percentage BS was estimated from pH values following Beery and Wilding (1971). Additionally, we quantified CEC and extractable nutrients using a Mehlich-3 extractant (Sikora & Moore, 2014).

We quantified baseline inorganic N concentrations by weighing ~6 g of fresh soil in centrifuge tubes and then adding 40 ml 2 M KCl. We then placed the tubes on a shaker for 1 h and centrifuged the tubes to separate the soil from the KCl. We poured the supernatant through a Whatman #42 filter and captured the filtered solution in a vial and then analyzed for baseline NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> analysis (Bundy & Meisinger, 1994) on an AlpKem Flow Solution IV automated wet chemistry system (O.I. Analytical).

## 2.5 | Biological soil properties

We measured potential mineralizable C by performing a 262-d incubation. We weighed 50 g of 8-mm sieved air-dried soil in plastic specimen cups and brought the soils up to 60% water-filled pore space by adding deionized H<sub>2</sub>O to the soil cups. Then we sealed the cups in a mason jar, stored then at 25 °C, and measured CO<sub>2</sub> concentrations using an infrared gas analyzer (LI-CIR 800, LI-COR Biosciences). We sampled each jar for CO<sub>2</sub> production and then flushed with CO<sub>2</sub>-free air periodically at 20 different times over the course of 262 d. The first four measurements took place biweekly followed by six weekly measurements, four measurements every 2 wk, three measurements every 3 wk, and finally three measurements every 4 wk. We then summed the CO<sub>2</sub> produced at each

sampling time point to determine the cumulative CO<sub>2</sub>. We weighed the soil cups at each CO<sub>2</sub> sampling throughout the incubation to make sure all soils maintained the same moisture content, and, if necessary, water was added to maintain 60% water-filled pore space.

We quantified potential NH<sub>4</sub><sup>+</sup> mineralization using an anaerobic incubation (Waring & Bremner, 1964). First, we combined ~6 g of fresh soil with 10 ml deionized H<sub>2</sub>O in a 50-ml centrifuge tube. We purged these tubes with N<sub>2</sub> and capped them for 7 d at 25 °C to promote mineralization and prevent nitrification. Next, we removed the caps and added 30 ml of 2 M KCl to each tube. We then placed the tubes on a shaker and analyzed as described above. We subtracted the 7-d NH<sub>4</sub><sup>+</sup> concentrations from the NH<sub>4</sub><sup>+</sup> baseline concentrations to determine potential NH<sub>4</sub><sup>+</sup> mineralization.

We assayed potential activities of six hydrolytic extracellular enzymes ( $\alpha$ -Glucosidase,  $\beta$ -Glucosidase, Cellobiohydrolase, and  $\beta$ -Xylodase, all of which are involved in C-acquisition; N-acetyl glucosaminidase, which is involved in N-acquisition; and acid phosphatase, which is involved in P acquisition) using the 96-well microplate fluorometric method (Bell et al., 2013; Koyama et al., 2013; Lynch et al., 2018; Wallenstein et al., 2009). Briefly, we combined 1 g of fresh soil with 30 ml of 50 mM sodium acetate buffer corrected for soil pH. Soil slurries were shaken for 1 h and then pipetted into a deep 96-well microplate. We pipetted fluorescing substrate for all substrates and incubated them for 3 h at 25 °C. We also prepared standards for each soil slurry using of 4-methylumbelliferone. When the incubation was complete, we centrifuged the plates and then transferred the sample from each well into black 96-well plates. Substrate fluorescence was measured on a Tecan Infinite M200 microplate reader (Tecan Trading AG). We also quantified PLFAs, which were extracted and analyzed following established methods (Denef et al., 2007; Gomez et al., 2014).

## 2.6 | Data analysis

We assessed the effect of grazing management on each soil property and farm pair with a general linear mixed-effects model (significant  $\alpha$  of  $p < .05$ ) using catena as a block and as a random effect. This was justified because catena represented the same soil type, slope, and aspect across each farm pair. Transect zones were treated as nested blocks within each catena block. We performed a combination of log and square root transformations when the data was nonnormal or had unequal variance. Additionally, we tested management and environmental differences (e.g., stocking rate, no. of paddocks, years under respective management, mean annual temperature and precipitation, soil type, texture, etc.) between farms as covariates, but none of these were significant and thus are not reported in this paper. The relative contribution

of each soil property was evaluated with principle component analysis to further determine grazing management differences. We used R software (v3.3.1; R Core Team, 2016) with lme4 package (Bates et al., 2015) and the factoextra package (Kassambara & Mundt, 2019).

### 3 | RESULTS

#### 3.1 | Physical soil properties

The average A-horizon depth overall was not significantly different from CG farms (Tables 1 and 2). Additionally, on average, AMP farms had slightly greater proportions of clay-sized particles, whereas CG farms had greater proportions of sand-sized particles (Tables 1 and 2;  $p$  value = .010). There were no significant differences in MWD or any individual aggregate size class across grazing management types or farm pairs (Table 2). Overall, we observed no significant differences in available WHC between AMP and CG farms (Table 2). However, in Pair 4, CG had greater WHC than AMP, and, in Pair 3, AMP had greater WHC than CG (Table 2).

On AMP farms, as we previously reported (Mosier et al., 2021), we found significantly more C within the DOM and MAOM fractions. The DOM C stocks were 18% greater on AMP farms (Figure 1a; Tables 1 and 2;  $p$  value = .031). The MAOM C stocks were nearly 2 Mg C ha<sup>-1</sup> greater on AMP farms than CG farms (Figure 1a; Tables 1 and 2;  $p$  value = .045). There was no significant difference between LPOM C or HPOM C stocks on AMP and CG farms (Figure 1a; Table 2).

#### 3.2 | Chemical soil properties

We found relatively large differences between grazing management types with respect to chemical soil properties. On average, AMP farms had 16% more total soil organic C in the A-horizon than CG farms as reported in Mosier et al. (2021) (Figure 1a; Tables 1 and 2;  $p$  value = .007). These higher stocks were found on four out of five of the AMP farms (Table 2). A-horizon total soil N stocks paralleled the findings of total soil organic C; however, AMP farms had 20% more total soil N than CG farms (Figure 1b; Tables 1 and 2;  $p$  value = .002; Mosier et al., 2021). Similar to total soil organic C, higher N stocks were found on four out of five of the AMP farms (Table 2). Initial inorganic N concentrations also varied across grazing management types. On average, CG farms had 29% greater NH<sub>4</sub><sup>+</sup> concentrations, whereas AMP farms had 55% greater NO<sub>3</sub><sup>-</sup> concentrations (Figure 2a; Tables 1 and 2;  $p$  value < .001 and  $p$  value = .015, respectively). Initial inorganic N concentrations varied between each farm pair (Table 2).

Other chemical soil properties also showed strong differences between grazing managements. There was significantly higher pH, BS, EC, and CEC on the AMP farms relative to CG farms. On average, AMP farms had an average pH of 5.9 vs. 5.6 on CG farms (Tables 1 and 2;  $p$  value < .001). The %BS, EC, and CEC were all greater on AMP farms than on the CG farms (Figure 3; Tables 1 and 2;  $p$  values = .001). These findings were observed on three of the five farm pairs but not necessarily on the same three farms (Table 2). And overall, we also found that three of the extractable nutrients we analyzed (Ca, Mg, and K) had greater concentrations on AMP farms than on CG farms (Table 2).

#### 3.3 | Biological soil properties

There was no difference between grazing managements in the total amount of mineralized C produced over the 262-d incubation (Tables 1 and 2). There was also no difference in the CO<sub>2</sub> respiration dynamics through time or between the cumulative CO<sub>2</sub> at any of the farm pairs (Tables 1 and 2). However, potential N mineralization was greater on CG farms than on AMP farms even when we normalized for soil N stocks. During our incubation, 60% more organic N was mineralized into NH<sub>4</sub><sup>+</sup> on CG farms (Figure 2b; Tables 1 and 2;  $p$  value = .008) and this result was consistent across all farm pairs but only statistically significant at two farm pairs (Table 2).

We found no other statistically significant differences in the biological soil properties measured. For example, there was no difference in overall extracellular enzyme activities for each enzyme type measured between grazing management types (Table 2). However, when we summed all the C acquiring enzymes, we found that on average, CG farms had 1.25 times more activity than AMP farms (Table 1). Additionally, CG farms had 1.32 times more N acquiring enzyme activity (and 1.25 times more phosphorus acquiring enzyme activity) (Table 1). We found no differences, and nearly identical values, between grazing management types in the PLFA microbial biomarker measurements both in total PLFAs and fungi/bacteria ratio (Tables 1 and 2).

#### 3.4 | Integrated soil property responses

We used principal component analysis with all soil properties to determine the relative contribution of each soil property to grazing management differences (Figure 4). Dimension 1 (DIM1) and Dimension 2 (DIM2) captured 22.6 and 14.6% of the variability in grazing management practices, respectively (Figure 4). The biggest contributors to differences between the two grazing managements were several chemical properties (BS, CEC, EC, and Ca and Mg concentrations) and total soil N stocks. The next biggest contributors were total

TABLE 2 General linear mixed-effects model (ANOVA) results (including all farm pairs) as well as farm pairwise comparison *p* values for each soil health indicator measured

A-horizon depth				Sand/clay ratio				Mean weight diam.				Available water holding capacity									
Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F						
df	df	value		df	df	value		df	df	value		df	df	value							
Grazing	1	10	.419	1	10	10.046	.010*	1	8.044	0.001	.976	1	10	1.160	.307						
Pair	4	10	.045*	4	10	46.735	<.001*	4	7.995	3.959	.046*	4	10	55.763	<.001*						
Grazing × pair	4	10	.912	4	10	2.970	.074	4	7.995	1.340	.335	4	10	14.071	<.001*						
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5						
Pr > F	.928	.358	.784	.661	.008	.031	.771	.078	.357	.463	.900	.307	.291	.163	.652	.061	.001*	<.001*	.582		
Dissolved organic matter				Light particulate organic matter				Heavy particulate organic matter				Mineral-associated organic matter									
Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F						
df	df	value		df	df	value		df	df	value		df	df	value							
Grazing	1	10	6.315	.031*	1	10	2.217	.168	1	10	3.944	.075	1	10	5.246	.045*					
Pair	4	10	14.447	<.001*	4	10	7.076	.006*	4	10	1.131	.396	4	10	2.365	.123					
Grazing × pair	4	10	5.973	.010*	4	10	0.328	.853	4	10	1.113	.403	4	10	0.578	.686					
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5						
Pr > F	.845	.165	.836	<.001*	.036*	.330	.637	.717	.998	.175	.807	.608	.608	.061	.350	.138	.855	.390	.094	.235	.227
Organic carbon stocks				Nitrogen stocks				Ammonium concentration				Nitrate concentration									
Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F						
df	df	value		df	df	value		df	df	value		df	df	value							
Grazing	1	10	11.266	.007*	1	10	17.085	.002*	1	10	34.814	<.001*	1	10	8.499	.015*					
Pair	4	10	3.688	.043*	4	10	5.355	.014*	4	10	14.256	<.001*	4	10	16.514	<.001*					
Grazing × pair	4	10	1.846	.197	4	10	1.584	.253	4	10	5.405	.014*	4	10	4.450	.025*					
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5						
Pr > F	.727	.070	.047*	.574	.014*	.847	.099	.084	.027*	.011*	.003*	<.001*	.067	.009*	.326	.763	.150	.160	.002*	.076	
pH				Electrical conductivity				Base saturation				Cation exchange capacity									
Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F	Num	Den	F	Pr > F						
df	df	value		df	df	value		df	df	value		df	df	value							
Grazing	1	10	25.666	<.001*	1	10	24.953	.001*	1	10	25.666	.001*	1	10	23.195	.001*					
Pair	4	10	26.305	<.001*	4	10	14.423	<.001*	4	10	26.305	<.001*	4	10	30.271	<.001*					
Grazing × pair	4	10	12.552	<.001*	4	10	3.008	.072	4	10	12.552	.001*	4	10	1.378	.309					
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5						
Pr > F	.012*	<.001*	.277	.005*	.227	.022*	.024*	.741	.001*	.455	.012*	<.001*	.277	.005*	.227	.455	.029*	.240	.004*	.036*	

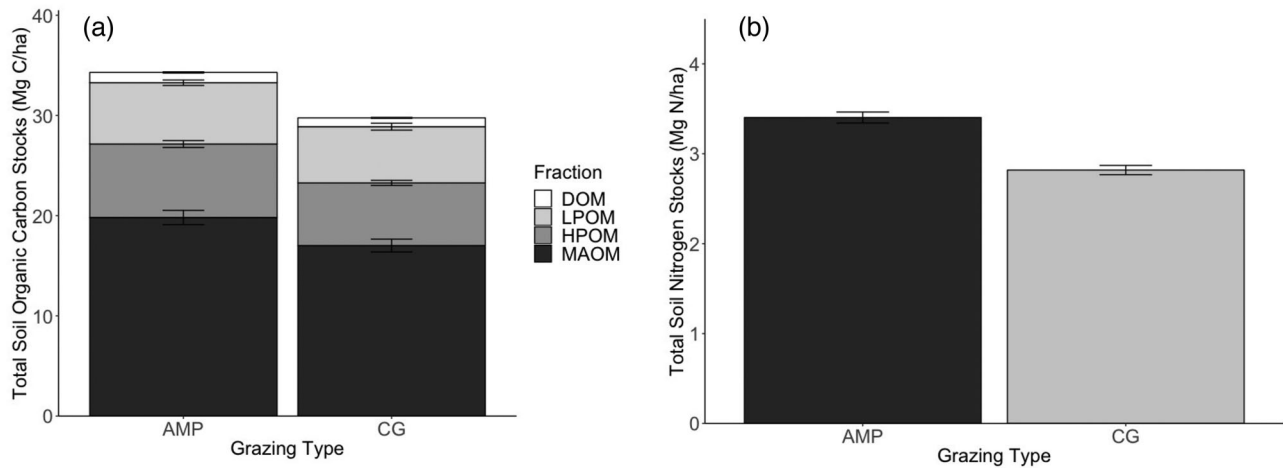
(Continues)

TABLE 2 (Continued)

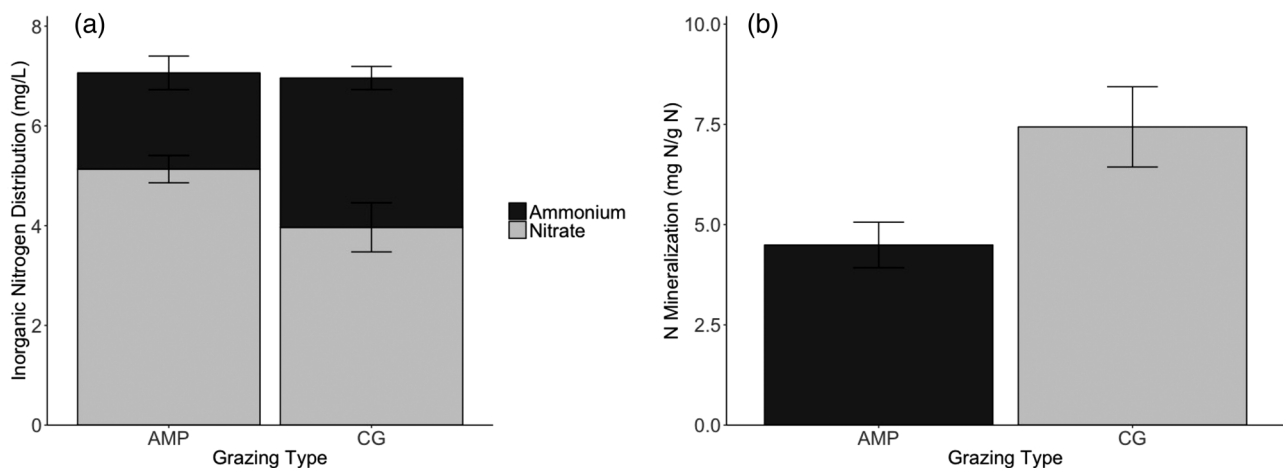
	Ca			Mg			K			Na										
	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F								
Grazing	1	10	61.095	<.001*	1	10	7.061	.024*	1	10	39.041	<.001*								
Pair	4	10	34.770	<.001*	4	10	15.177	<.001*	4	10	2.657	.096								
Grazing X pair	4	10	11.184	.001	4	10	4.591	.023*	4	10	12.100	<.001*								
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5					
Pr > F	.145	<.001*	.228	<.001*	.690	.059	.047*	.319	.006*	.160	<.001*	.988	.273	<.001*	.815	.008*	.021*	.691	.196	.893
	<b>Fe</b>			<b>Zn</b>			<b>Mn</b>			<b>S</b>										
	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F								
Grazing	1	10	3.007	.114	1	10	0.684	.428	1	10	0.345	.570								
Pair	4	10	44.402	<.001*	4	10	4.313	.028*	4	10	17.268	<.001*								
Grazing X pair	4	10	7.251	.005*	4	10	0.157	.956	4	10	1.786	.208								
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5					
Pr > F	.804	.008*	.147	.003*	.141	.545	.857	.887	.664	.435	.271	.069	.991	.263	.467	.045*	.918	.020	.667	.008*
	<b>Cu</b>			<b>C mineralization</b>			<b>N mineralization</b>			<b>C enzymes</b>										
	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F								
Grazing	1	10	3.322	.098	1	10	0.483	.503	1	10	10.892	.008*								
Pair	4	10	4.533	.024*	4	10	1.209	.366	4	10	12.105	<.001*								
Grazing X pair	4	10	6.660	.007*	4	10	1.646	.238	4	10	2.450	.115								
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5					
Pr > F	.210	.033*	.004*	.018*	.542	.808	.986	.981	.313	.036*	.753	.028*	.962	.448	.004*	.305	.125	.467	.419	.271
	<b>N enzymes</b>			<b>Phosphorus enzymes</b>			<b>Total phospholipid fatty acids</b>			<b>Fungi/bacteria phospholipid fatty acids</b>										
	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F	Num df	Den df	F value	Pr > F								
Grazing	1	10	1.299	.281	1	10	2.051	.183	1	10	0.057	.817								
Pair	4	10	2.009	.169	4	10	1.993	.172	4	10	8.893	.003*								
Grazing X pair	4	10	0.676	.624	4	10	1.747	.216	4	10	0.846	.527								
Farm pairwise comparison	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5					
Pr > F	.415	.326	.473	.900	.229	.145	.062	.301	.386	.769	.921	.218	.341	.913	.431	.491	.400	.263	.520	.848

\*Significant at the .05 probability level.

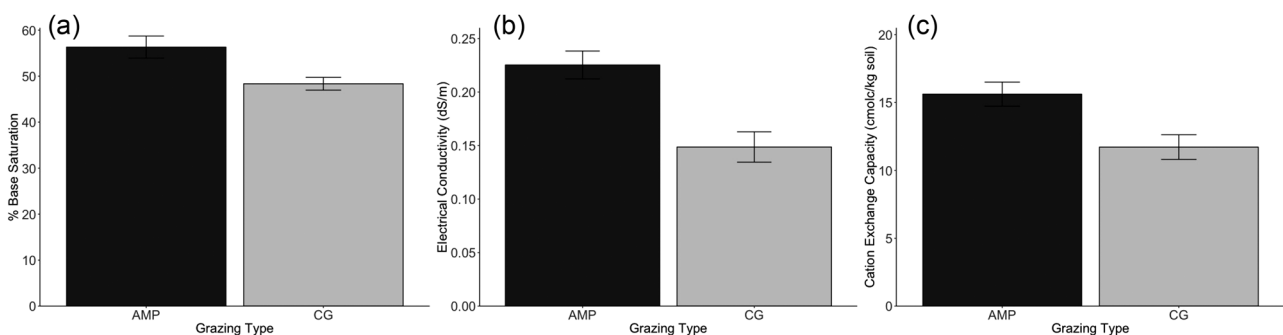




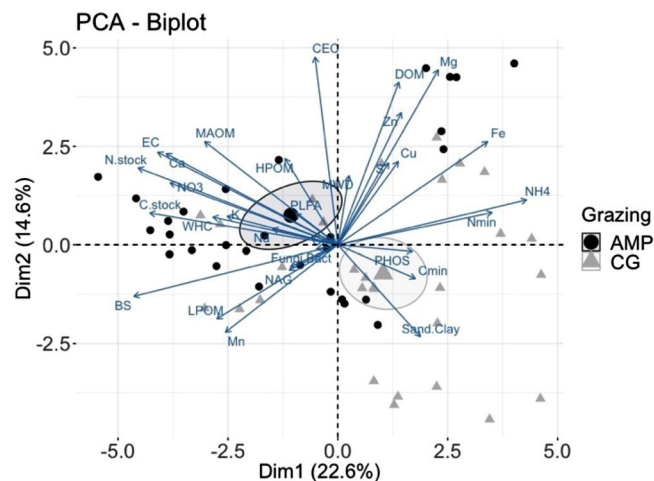
**FIGURE 1** Comparison of (a) separated soil organic matter fraction carbon stocks ( $\text{Mg C ha}^{-1}$ ) and dissolved organic matter (DOM), light particulate organic matter (LPOM), heavy particulate organic matter (HPOM), and mineral-associated organic matter (MAOM); and (b) total soil nitrogen stocks ( $\text{Mg N ha}^{-1}$ )  $\pm$  SE between adaptive multipaddock grazing (AMP) and conventional grazing (CG) farms. Data are a subset from Mosier et al., 2021)



**FIGURE 2** Average (a) inorganic nitrogen concentrations ( $\text{mg N L}^{-1}$ ) and (b) potential nitrogen mineralization ( $\text{mg N g soil N}^{-1}$ )  $\pm$  SE between adaptive multipaddock grazing (AMP) and conventional grazing (CG) farms



**FIGURE 3** Comparison of average (a) percentage base saturation, (b) electrical conductivity ( $\text{dS m}^{-1}$ ), and (c) cation exchange capacity ( $\text{cmolc kg soil}^{-1}$ )  $\pm$  SE between adaptive multipaddock grazing (AMP) and conventional grazing (CG) farms



**FIGURE 4** Principle component analysis of all measured soil health indicators contribution to the differences between adaptive multipaddock grazing (AMP) and conventional grazing (CG) farms. The relative contribution of each indicator is reflected in the length and the direction of the arrows. The data points are color coded by grazing management with ellipses representing 95% confidence intervals. Acronyms are as reported in Table 1

soil organic C stocks, MAOM C stocks, DOM C stocks, and  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and Fe concentrations. The lowest contributors were the biological properties (PLFAs, extracellular enzyme activities, and C mineralization).

## 4 | DISCUSSION

Using Tier 1 and Tier 2 proposed soil properties from the Soil Health Institute (Norris et al., 2020), overall, AMP soils had better soil chemical properties in comparison to CG farms. While many of the physical properties and most of the biological properties were similar between grazing management types, the SOM fraction distribution and most of the chemical properties were significantly improved in AMP soils. Moreover, AMP grazing management did not negatively affect the soil environment in any of the soil properties measured with the exception of potential N mineralization. In addition, the increased nutrient retention and availability under AMP grazing management compared with CG management, evidenced by improved chemical properties, SOM fractions, and C and N stocks, demonstrates that AMP management practices could be implemented to improve components of grassland soil health across a large area of grazing lands in the southeastern United States.

Our results showed that AMP soils had similar soil structure as CG soils with no significant differences in A-horizon depth and similar aggregation, which resulted in no differences in average MWD between grazing managements. Similar amounts of aggregation between AMP and CG farms

tells us that having the cattle in higher stocking densities on AMP farms does not negatively affect aggregate stability or the MWD of soil aggregates, and there will still be significant structure in the soil. Had we measured WHC on intact soil cores, rather than disturbed samples that were ground, we may have seen differences in WHC between grazing managements. Because of the depth of the A-horizon analyzed, we would expect to see differences in percentage clay even though these sites are on similar soil types simply because more silt and clay are typically found deeper in the soil profile (Keen & Rackowski, 1921). So, it is not surprising that we found a higher proportion of clay on the sites with a deeper A-horizon.

As we previously reported (Mosier et al., 2021), the majority of the physical SOM fractions stored more C in the A-horizons of AMP soils than in CG soils, apparent in DOM C, MAOM C, and marginally in HPOM C. We observed no difference in LPOM C between grazing managements, which suggests that structural plant inputs to the system are similar. Yet cycling of C may be more efficient in AMP soils as they had greater DOM, MAOM, and total soil organic C stocks. Additionally, the increase in total soil N stocks indicates more retention of N in the AMP soils, even though the AMP farms in this study do not use N fertilizers (Mosier et al., 2021). So, taken together, a reduction in fertilizer use and an increase in N retention have the potential to decrease  $\text{N}_2\text{O}$  emissions on AMP farms. An increase in N retention is also likely contributing to the formation of more MAOM on AMP farms because N stocks can lead to greater C use efficiency and ultimately higher MAOM stocks (Cotrufo et al., 2013, 2015; Averill & Waring, 2018).

We found similar amounts of biological activity and microbial PLFA biomarkers across AMP and CG farms. However, we saw slightly greater enzymatic activity on the CG farms than on the AMP farms, although these activities were highly variable. Extracellular enzyme activity results are often difficult to interpret because it is impossible to say whether microbes are producing enzymes to acquire nutrients or if they are producing enzymes because those nutrients are easily available (Wallenstein et al., 2012; Bell et al., 2013). In this case, the results suggest that the microbial community might be producing more enzymes in the CG soils in order to acquire the resources that are less abundant than in AMP soils. This finding is also evidenced by the greater potential N mineralization rates and greater soil  $\text{NH}_4^+$  concentrations on CG farms than on AMP farms. Because there is less N available to microbes on CG farms, microbes likely need to mineralize more organic matter to obtain more inorganic N. However, the lack of a significant biological response to grazing management suggest that these properties may not be the most robust metrics for capturing management differences and possibly other biological properties may be more appropriate such as other measures of diversity (Lehmann et al., 2020).

There were no differences in the specific potential C mineralization between farms. Carbon mineralization measurements are often used as proxies for microbial activity. Therefore, this shows that any increase in soil C in the AMP soils has not increased the total or relative amount of mineralizable C (i.e., the amount of C respired per unit of soil C was the same across AMP and CG soils). This highlights that even with more C present at the AMP farms, the microbial community is not stimulated to respire more CO<sub>2</sub> per gram of C and could possibly be using the available C more efficiently, further evidenced by AMP soils having significantly more MAOM C stabilization than CG soils.

Some of the biggest differences between grazing management practices was found in the chemical soil properties. Typically, EC is used as a measure of salinity and is considered an important soil property because high levels of salt can be detrimental for vegetation growth (Smith & Doran, 1996). None of our farms were close to EC values that may be considered detrimental to plant growth. Having higher EC within an acceptable range for vegetation growth can have its advantages, as it can influence the way in which cations (i.e., essential nutrients) move through the soil profile (Smith & Doran, 1996). Percentage BS, pH, and CEC are indicators of nutrient retention and availability (Smith & Doran, 1996; Cornell University Cooperative Extension, 2007). Our AMP farms seem to be more capable of holding onto nutrients and supplying plant-available nutrients based on the greater extractable nutrients, pH, percentage BS, and CEC. Of the extractable nutrients measured, only Ca, K, and Mg had significantly greater concentrations on the AMP farms. These key nutrients are also some of the extractable nutrients used by plants in the greatest quantities (Cornell University Cooperative Extension, 2007). Calcium is crucial for plant growth and development and is also crucial in helping to regulate soil acidity (Rengel, 2002). Potassium helps plants use N and water efficiently (Baligar et al., 2001) but can be easily leached out of sandier soils with low CEC (Cornell University Cooperative Extension, 2007), which is likely why significantly less was found on CG farms. Magnesium and Zn were also elevated on the AMP farms compared with the CG farms and are important for plant metabolism processes such as photosynthesis (Bolan et al., 2002) as well as enzyme and protein synthesis (Lindsay, 1972). Taken together, the PCA results, in combination with the mixed-effects model ANOVA results, the chemical properties seem to be very important contributors to the differences in soil health across the two grazing management practices.

## 5 | CONCLUSIONS

The number of soil properties that were improved where AMP grazing management was implemented (e.g., chemical

properties, SOM fraction distribution, and total soil organic C and N stocks) highlights the potential of AMP grazing management to better supply and retain essential nutrients for plant productivity. And when soil properties were not improved, they were almost always similar between grazing management types and not lowered on AMP grazing farms with the exception of potential N mineralization. These results also point to chemical soil properties coupled with SOM fraction distribution as important indicators to detect grazing management changes on soil health. Overall, these findings imply that farms implementing AMP grazing management will be better equipped to support grazing operations while also maintaining a sustainable soil environment. These results provide evidence that AMP grazing management could be used as a way to improve grassland soil health across a large area of currently conventionally managed grazing lands.

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## AUTHOR CONTRIBUTIONS

Samantha Mosier: Data curation; Formal analysis; Investigation; Visualization; Writing – original draft. Steve Apfelbaum: Conceptualization; Funding acquisition; Writing – review & editing. Peter Byck: Conceptualization; Funding acquisition; Supervision; Writing – review & editing. Jim Ippolito: Data curation; Investigation; Methodology; Writing – review & editing. M. Francesca Cotrufo: Conceptualization; Investigation; Project administration; Supervision; Writing – review & editing.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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