Adaptive multi-paddock grazing enhances soil carbon and nitrogen stocks and stabilization through mineral association in southeastern U.S. grazing lands

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Abstract

Grassland soils are a large reservoir of soil carbon (C) at risk of loss due to overgrazing in conventional grazing systems. By promoting regenerative grazing management practices that aim to increase soil C storage and soil health, grasslands have the potential to help alleviate rising atmospheric CO_2 as well as sustain grass productivity across a vast area of land. Previous research has shown that rotational grazing , specifically adaptive multi-paddock (AMP) grazing that utilizes short-duration rotational grazing at high stocking densities, can increase soil C stocks in grassland ecosystems, but the extent and mechanisms are unknown. We conducted a large-scale on-farm study on five "across the fence" pairs of AMP and conventional grazing (CG) grasslands covering a spectrum of southeast United States grazing lands. We quantified soil C and nitrogen (N) stocks, their isotopic and Fourier-transform infrared spectroscopy signatures as well as their distribution among soil organic matter (SOM) physical fractions characterized by contrasting mechanisms of formation and persistence in soils. Our findings show that the AMP grazing sites had on average 13% (i.e., 9 Mg C ha^{-1}) more soil C and 9% (i.e., 1 Mg N ha^{-1}) more soil N compared to the CG sites over a 1 meter depth. Additionally, the stocks' difference was mostly in the mineral-associated organic matter fraction in the A-horizon, suggesting long-term persistence of soil C in AMP grazing farms. The higher N stocks and lower ^{15}N abundance of

AMP soils also point to higher N retention in these systems. These findings provide evidence that AMP grazing is a management strategy to sequester C in the soil and retain N in the system, thus contributing to climate change mitigation.

1. Introduction

Grasslands hold a large amount of soil organic matter (SOM) averaging up to 173 Mg C ha^{-1} down to a 1 meter depth in U.S. temperate grasslands (Schlesinger, 1977). Grasslands also extend over vast areas in the U.S. and those that are grazed by large ungulates cover over 30% of total U.S. land (Bigelow and Borchers, 2012). Grassland management improvements have been identified as a climate change mitigation strategy that could have a high impact due to its large potential area of adoption, sequestering up to 0.3 to 1.6 Pg CO_2 eq. per year (Paustian et al., 2016). Optimizing grazing intensity is also expected to have significant ecosystem services co-benefits to soil carbon (C) sequestration, such as reduced disturbance to plant-insect interactions and reduced water use (Bossio et al., 2020), and increased nitrogen (N) retention (de Vries et al., 2012).

Under conventional grazing (CG) management, stocking rate is the management control variable that attempts to align forage availability with animal forage requirements and animals are continuously left in an area or are infrequently rotated. When left free to graze, cattle tend to congregate in areas with nutritious forage and deplete forage quickly (Teague et al., 2013; Barnes et al., 2008). These areas will thus experience high rates of erosion, bare patches of ground, and have a harder time regenerating (Teague et al., 2016; Teague et al., 2004; Bailey et al., 1998). Such overgrazing from CG management has led to losses of soil C and ecosystem function from grasslands (Teague, 2018; Conant and Paustian, 2002; Conant et al., 2001) as well as altered N cycling, increased erosion, and runoff of plant available water and nutrients (Piñeiro et al., 2010; Milchunas and Laurenroth, 1993).

Conversely, several types of rotational grazing can restore grassland ecosystem function and have beneficial impacts on soils as well (Conant et al., 2003; Dubeux et al., 2006; Teague et al., 2011; Machmuller et al., 2014; Teague, 2018; Stanley et al., 2019). However, many of the studies examining rotational grazing were performed on a smaller scale, with different levels of management and animal rotation, which produced results of varying magnitudes and directions (Briske et al. 2008, Teague et al., 2015). This has made it difficult to understand where and when improvements in management will have significant benefits to the soil environment. Rotational grazing is a broad term that is defined in many ways and can include systems ranging from 2 to 40+ paddocks, which can greatly influence the level of animal movement and management intensity. Some rotational grazing systems can be prescriptive with regularly planned animal movements or more flexible, where animal movements are based on available forage growth (Undersander et al., 2002). In this research, we focus on adaptive multi-paddock (AMP) grazing, a form of short-duration rotational grazing at high stocking densities that some farmers have adopted with the goal of increasing soil and plant health and animal well-being.

AMP involves using multiple fenced paddocks, which are grazed for short periods (hours to days, depending on the season), during which plant consumption is monitored (aimed to leave ~50% forage uneaten), followed by an adequate time of recovery after grazing to allow vegetation regrowth (Teague et al., 2013). AMP grazing management adjusts livestock numbers to not exceed available forage and to avoid overstocking and overgrazing. Additionally, AMP practitioners seek to minimize the use of external inputs (i.e., fertilizers, herbicides, pesticides).

Among other benefits, AMP grazing can increase biodiversity, plant nutrition and cow health (Teague et al., 2016). However, we currently lack information on the effect of AMP grazing at large scales, and on the C distribution across different SOM fractions and soil depths. This points to the need for studies comparing AMP with CG management, across differing soil types, environmental conditions, and assessing soil C changes over soil depths and beyond the bulk soil, in functionally distinct SOM fractions.

Soil C exists in a variety of chemical and physical forms, and to fully understand soil C responses to management, it is important to separate bulk soil C into functionally distinct fractions (Lavallee et al., 2020), which form and stabilize through distinct pathways (Cotrufo et al., 2019; Cotrufo et al., 2015). Four SOM fractions with distinct properties and functions are: (1) light particulate organic matter (light POM; <1.85g cm^{-3}), made of partly decomposed plant, and to a lesser extent, microbial structural residues (Christensen, 2001); (2) heavy sand-sized POM (heavy POM; >1.85b cm^{-3} and >53μm) made of more decomposed plant and microbial compounds coating sand-sized particles and often protected by aggregates (Golchin et al., 1997); (3) dissolved organic matter (DOM), made of readily bioavailable low-molecular weight soluble or suspended compounds derived from labile plant inputs, root exudates, and microbial metabolites (Kalbitz et al., 2000), which can exchange with (4) mineral-associated organic matter (MAOM; >1.85g cm^{-3} and <53μm), made of low-molecular weight compounds and microbial extracellular polymeric structures chemically bonded to minerals (Kleber et al., 2015). Separating and quantifying SOM into these functionally meaningful fractions increases the power of detection of C stock changes while providing more information about the mechanisms driving SOM accrual, its persistence, and vulnerability to disturbance and management practices.

Our study evaluated the effect of grazing management on soil C and N storage across the southeast region of the United States, by comparing AMP grazing with CG management. We analyzed soils from five paired, neighboring AMP and CG farms located on grasslands in the southeast United States. Based on previous research, we expected that, with higher cattle stocking densities combined with adequate pasture rest time and vegetation regrowth (Table 2), AMP grazing management would have higher soil C and N stocks. In turn, we expected the increases in soil N to result in higher MAOM formation (Cotrufo et al., 2013; Averill and Waring, 2018), and overall increased N retention in AMP grazing relative to CG management.

2. Methods

2.1 Study Sites

Study sites represented a latitudinal gradient from Adolphus, Kentucky through Woodville, Mississippi (Table 1). The AMP and paired, adjacent CG managed farms were selected through a careful screening process. First, we used an online survey which was created with input from the regional Natural Resource Conservation Service agency (USDA-NRCS) as well as other grazing organizations (i.e., GrassFed Exchange) to identify AMP farmers in our region of interest. Ninety farmers claiming to practice AMP grazing completed the survey. We selected 25 farms for in-person visits, based on their self-reported management practices. We focused on specific management criteria including: stocking rates, number of paddocks, animal movement frequency, paddock recovery times, legacy of fertilization, liming, and herbicide use, and length of management history. We then searched for a potential CG neighboring farm grazing on areas under the same soil type and aspect as the perspective AMP farms (Table 1; Supplemental Figure 1), and with a similar land use history (Table 2). The final selection of the most representative five pairs of neighboring AMP and CG farms was based on the farms that most closely represented our definition of AMP grazing with a neighbor practicing CG, which is the most common and representative grazing management in the region based on county averages (Table 2).

We used the amount of paddocks as the key definer of management practice, influencing amount of rest days, as well as stocking densities. Specifically in this study, the AMP treatment had >40 paddocks, stocking rates > 1 animal unit ha^{-1}, stocking densities > 60 animal unit ha^{-1}, and a rest:grazed day ratio of >40 days, while the CG treatment had values below these thresholds (Table 2). This resulted in a clear management separation between the selected CG and AMP farms (Supplementary Figure 2). Interestingly, conventional practices are much more similar among them, while AMP grazing being “adaptive” by definition spans a broader range of practices. We also confirmed that each neighboring pair had one or two pastures on the same soil type to allow for valid comparisons by testing preliminary soil cores in the field and mid-infrared spectroscopy (MID-IR) analysis (Table 1; Supplementary Figure 1). On the other hand, the five pairs provided a broad range of soil types common to the southeast U.S. region (Table 1).

2.2.Soil sampling and processing

Our soil sampling followed the VM0021 “Soil Carbon Quantification Method” which is approved for the carbon marketplace (Verra, 2011). At each grazed farm, we sampled two representative catenas on the identified common soil type using three sampling zones (~10-

30m in width) representing either the upper, middle, and lower slope position of the catena (Supplemental Figure 3). Within each sampling zones, we randomly chose seven soil sampling locations. Each of the seven soil cores were collected with an ATV mounted Giddings hydraulic sampling unit to a 1 meter depth (average core depth of 85cm). The cores were 5cm in diameter and were extracted using direct push with no turning or torsional compaction risk that would impact bulk density. We extracted core samples in plastic sleeves for a total of 42 cores per farm and 420 cores total (Supplemental Figure 3). All soil sampling occurred in May-June 2018.

Cores were delivered to Colorado State University in protective crates where they were stored at 5°C until they were processed within four weeks of arrival. Processing began by making sure there was no soil compression during transport. Soil core lengths were documented in the field and were checked to make sure the cores were the same length upon delivery. We next separated each core into horizons and depth increments by recording the depth and extracting the A-horizon using a knife. Then we extracted the depth increments below the A-horizon to 30cm, 30-50cm, and 50-100cm. We sieved each soil sample through 8mm wire mesh, and removed rocks, roots, and noticeable litter, which were oven-dried and weighed. A representative soil sample from each depth increment was measured for gravimetric water content and the remaining sample was de-quarantined by heat treatment in a 110°C oven, according to USDA APHIS protocol. After heat treatment, we sieved the soils through 2mm wire mesh, and any remaining rock, root, and litter fragments were removed, cleaned of any dried soil, and quantified. The weight of the removed materials was used to adjust bulk density and estimate standing root mass at the time of sampling, which were determined using the core method for each core depth increment (Mosier et al., 2019). We did not have the resources to analyze all root biomass samples for %C. Therefore, we obtained standing root C stocks by applying 45% C average estimates to the observed root mass (Ma et al., 2018).

2.3 Soil elemental and isotopic analyses

To determine total soil C and N concentrations and $\delta^{13}C$ and $\delta^{15}N$ natural abundances we ground and analyzed a subsample of the 2mm sieved, oven-dried soil by dry combustion on a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA) coupled with a Delta V Advantage isotope ratio mass spectrometer (Thermo-Fisher, Bremen, Germany). We also tested the soils for the presence and amount of inorganic C using an acid pressure transducer connected to a voltage meter (Sherrod et al., 2002). Inorganic C concentrations were generally negligible, but if any was found, it was removed from the total C amount to allow us to determine total organic C. Total organic C and N stocks were determined by sample, using C and N concentrations and bulk density measurements.

2.4 MID-IR spectrometry analyses

We characterized all soils chemically by MID-IR to verify that soils from paired farms were fundamentally similar and had the same underlying minerology. Only A-horizon spectra are reported here, since the spectra at depth did not provide any additional relevant information. Additionally, we compared the organic band spectra of the A-horizon between paired soils, to identify if grazing management had any specific effect on SOM chemistry. We focused on the A-horizon because this is where most of the plant and soil biological activity occurs (Scott and Moebius-Clune, 2017) and therefore expected it to be the most sensitive to chemical changes from grazing management. Soils were analyzed using a Digilab FTS 7000 spectrometer (Varian, Inc., Palo Alto, CA, USA) with a Pike AutoDIFF diffuse reflectance sampler (Pike Technologies, Madison, WI, USA) for spectral analysis. The MID-IR (4000-400 cm^{-1}) pseudo absorbance was obtained using a KBr background and deuterated triglycine sulfate detector. Each spectrum was made of 64 co-added scans and 4 cm^{-1} resolution. Organic band assignments were informed by Parikh et al. (2015).

2.5 Soil organic matter fractionation

The 2mm sieved samples were composited by sampling zone to create a representative sample for each sampling zone at each depth. There were six sampling zones per grazed farm for a total of 60 zones, and four representative depths for each zone. This gave us a total of 240 composited samples for the SOM fractionation analysis (Supplemental Figure 3). We fractionated each composited sample similar to Mosier et al. (2019), but modified to sample for the DOM fraction and to disperse aggregates prior to the separation of light POM, heavy POM, and MAOM. Briefly, we added DI H_2O to 6g of 2mm oven-dried composited soil and shook for 15 minutes, then centrifuged for 15 minutes at 3400 rpm. Then we poured off the DOM fraction and analyzed for total organic C and total N on a Shimadzu TOC-L/TNM-L Analyzer (Shimadzu Corporation, Kyoto, Japan). To the remaining soil, we added sodium polytungstate (1.85g cm^{-3}) and dispersed aggregates by reciprocal shaking for 18 hours. After dispersion we centrifuged the sample for density fractionation and aspirated the light POM (<1.85g cm^{-3}) from the rest of the soil. We then thoroughly rinsed the residual soil and separated into heavy POM (>53µm) and MAOM (<53µm) by wet sieving. All fractions were analyzed for %C and %N on an elemental analyzer as described above for the bulk soils.

2.6 Data analyses

We assessed the effect of grazing management type and pair location on %C, %N, bulk density, total soil organic C and N stocks, $\delta^{13}C$ and $\delta^{15}N$ soil signatures, as well as the distribution of each SOM C stock between functionally distinct fractions (DOM, light POM, heavy POM, and MAOM) with a general linear mixed-effects model using a significant alpha level of $p<0.05$. Grazing management type, farm pair, as well as their interaction were treated as fixed effects. We accounted for our sampling design by using a nested block as one random effect in our model (sampling zone nested within catena). This allowed us to look at the overall effect of grazing management type across all farm pairs as well as the differences in grazing management type between each farm pair while accounting for any variability between each catena and sampling zone. The 1 meter deep C and N stock data were calculated using only 377 cores (rather than all 420) because some of the soil cores did not reach past the 50cm depth. Additionally, some SOM fractions and isotope values were left out of the analysis because some samples had too little material to get accurate data from the elemental analyzer. The exact sample numbers are reported in each figure legend. R software was used for all analyses (R version 3.3.1; R core Team, 2016) with the lme4 package (Bates et al., 2015) and the factoextra package (Kassambara, 2019).

We performed a combination of log and square root transformations when the data was non-normally distributed or had unequal variance. We tested factors associated with management (i.e., number of paddocks, fertilization, stocking density) and the environmental differences (i.e., MAP, MAT, soil type) among farms as covariates (Table 1 and Table 2). We ultimately left out all of the covariates from the final model as none of the management or environmental factors were significant nor did they confound our main model effects. All covariate information was either collected by us and other project partners through on the ground measurements, farmer interviews and surveys, or derived from local climate stations.

3. Results

3.1 Total soil organic carbon stocks

On average, there was 13% more total soil organic C to 1 meter depth on AMP farms compared to CG farms. The average total soil organic Mg C ha^{-1} ± standard error on AMP farms was 72.49 ± 1.25 while CG farms had on average 64.02 ± 1.04 (Figure 1a; $p=0.02$). Across all pairs, the increase in soil organic C stocks was most pronounced in the A-horizon depth, but was significantly higher at each depth increment down to 50cm (Figure 1a; Supplemental Table 1). Individual farm pairs varied from 4% lower to 22.75% greater soil C stocks (Figure 2a). There was only one farm pair (Pair 4) where CG had greater soil organic C stocks compared to AMP, and another farm pair (Pair 1) where AMP had higher soil organic C stocks compared to CG, yet in both cases differences were not statistically significant (Figure 2a; Supplemental Table 2). The other three farm pairs had significantly more soil organic C under AMP grazing than CG (Figure 2a; Supplemental Table 2).

3.2 Total standing root carbon stocks

Overall, standing root mass C stocks were relatively small compared to the total soil organic C stocks (Figure 2b). Across all farm pairs, there was significantly more total standing root mass C (Mg C ha^{-1} ± standard error) on CG farms compared to AMP farms (6.99 ± 0.40 and 3.30 ± 0.23 respectively, $p=0.01$). This result was driven by the two southernmost farm pairs (Pairs 4 and 5), where on average CG farms had over four times more standing root biomass C than AMP farms. In the other three farm pairs, there was no difference in root biomass C between grazing types (Figure 2b). When standing root biomass C and total soil organic C were combined and averaged across farm pairs, AMP farms still had greater total belowground C (Mg C ha^{-1} ± standard error) than CG farms (75.79 ± 1.31 and 71.01 ± 1.09 respectively; p= 0.04).

3.3 Total soil nitrogen stocks

Total soil N stocks (Mg N ha^{-1} ± standard error) were significantly greater in AMP farms (9.26 ± 0.14) relative to CG farms (8.52 ± 0.13) (Figure 1b; $p<0.01$). Along the 1 meter depth, there was on average over 8% more soil N in AMP farms compared to CG farms (Figure 1b). These differences in soil N stocks were most prominent in the A-horizon, but they were still significantly higher at each depth increment down to 50cm (Figure 1b; Supplemental Table 1). Total soil N stocks were consistent for all five farm pairs, with AMP farms having 7.8% to 12.39% greater soil N stocks than CG, but were only statistically significant on three farm pairs (Figure 2c).

3.4 Bulk density, %C and %N

Differences in soil organic C and N stocks were the result of differences in C and N concentrations, not bulk density. We found no significant differences in bulk density between grazing managements at any of the core depth increments except in the 50-100cm depth. Only in the 50-100cm depth did the CG farms have on average higher bulk density (1.51g cm^{-3}) than the AMP farms (1.44g cm^{-3}; p=0.014; Supplemental Table 1). We measured significantly higher C and N concentrations on AMP farms compared to CG farms at every core depth increment except the 50-100cm depth (Supplemental Table 1). At this deepest depth, we found no differences in either C or N concentrations.

3.5 Soil organic matter fraction carbon

Soil organic C distribution shifted towards the MAOM fraction in AMP farms at all soil depth increments measured. Overall, there was 25% more C in the MAOM fraction on AMP farms compared to CG farms, with average MAOM C stocks (Mg C ha^{-1} ± standard error) of 56.14 ± 1.98 in AMP and 44.82 ± 1.01 in CG farms (Figure 3; Supplemental Table 2; $p<0.01$). Additionally, there was 15% more C in the heavy POM fraction on AMP farms compared to CG farms, with AMP having 9.80 ± 0.36 and CG having 8.47 ± 9.27 Mg C ha^{-1} in heavy POM (Figure 3; Supplemental Table 2; $p=0.02$). Similarly, we found significantly more DOM C in the AMP farms compared to the CG farms (2.50 ± 0.13 compared to 2.19 ± 0.14, respectively) however, this fraction only contributed 3% of the total soil C (Figure 3; Supplemental Table 2; $p<0.01$). In contrast, there were no overall differences in the amount of C found in the light POM fraction, with the exception of Pair 5, where AMP had significantly more light POM than CG (Figure 3; Supplemental Table 2). Overall, farm location was not a significant factor in the general linear mixed-effects model for any of the SOM fractions besides DOM (Supplemental Table 2), suggesting a generalizable response of C distribution across SOM fractions to grassland management.

3.6 Soil organic matter fractions C:N ratios

Soil N distribution across SOM fractions generally followed the C distribution. However, the AMP farms had a lower C:N ratio in the A-horizon of the bulk soil and several of the SOM fractions when compared to CG farms. Across all pairs, there were no differences in the A-horizon C:N ratio of the MAOM fraction (Supplemental Table 3), but the A-horizon C:N ratio (average ± standard error) was lower in the heavy POM (13.18 ± 0.43 compared to 14.91 ± 0.43, p=0.02), light POM (15.87 ± 0.48 compared to 18.00 ± 0.48; p=0.04), and DOM fractions (7.36 ± 0.33 compared to 8.02 ± 0.40; p=0.04) on AMP farms relative to CG farms (Supplemental Table 3). This trend of lower fraction C:N continued down to 50cm, but the differences were much less pronounced and not statistically significant below the A-horizon (Supplemental Table 3).

3.7 Natural abundance soil $\delta^{13}C$ and $\delta^{15}N$ values

We measured lower natural abundance $\delta^{13}C$ signatures in AMP bulk soils at all depths compared to CG bulk soils. However, this was only significant in the top two depth increments (Figure 4a; p<0.01). This result was consistent across all farm pairs in the A-horizon depth and all but Pair 2 in the bottom of A-horizon to 30cm depth (Supplemental Table 4). AMP bulk soil $\delta^{13}C$ values ranged from -21.6 to -23.2 whereas CG bulk soil $\delta^{13}C$ values ranged from -20.8 to -21.2 (Figure 4a). Additionally, we found a trend of lower natural abundance $\delta^{15}N$ signatures in AMP bulk soils at all depth compared to CG bulk soils. This finding was only significant from the bottom of the A-horizon to 30cm and the 30-50cm depth increments (Figure 4b; p<0.01) and varied across farm pairs (Supplemental Table 4). For example, we found no significant differences in $\delta^{15}N$ values between AMP and CG in soils from Pairs 1 and 5, whereas the other three pairs had significantly lower $\delta^{15}N$ values on the AMP farms (Supplemental Table 4). AMP bulk soil $\delta^{15}N$ values ranged from 3.7 to 5.2 whereas CG bulk soil $\delta^{15}N$ values ranged from 4.1 to 6.2 (Figure 4b).

3.8 MID-IR spectroscopy

Overall, the paired AMP and CG soils had very similar spectral features with the same underlying minerology as revealed by the MID-IR spectra (Supplemental Figure 1). On the other hand, spectra were different across pairs, confirming that we spanned a broad range of soils from the southeast U.S. region in our study (Table 1).

In order to identify if grazing management had induced any changes in the chemical features of SOM, we performed spectral subtractions by pair to isolate specific organic spectral features of AMP grazing as compared to CG management (Figure 5). Pair 3 had the greatest response to AMP management in terms of total belowground C and heavy POM (Supplemental Table 2). For this pair, the AMP bulk soil spectra had higher absorbance than the CG bulk soil at several bands: 2930-2850 cm^{-1} (Aliphatic C-H), 1690 cm^{-1} (C=O stretch), 1610 cm^{-1} (Unassigned), 1520 cm^{-1} (Aromatic C=C, or amide N-H), 1250 cm^{-1} (Possibly carboxylic acid C-O), and 1160 cm^{-1} (C-OH stretch, attributed to polysaccharides). Pairs 2, 4, and 5 had greater MAOM C in AMP soils compared to CG soils (Supplemental Table 2). In these pairs, the MID-IR spectra of the AMP bulk soils had higher absorbance at different points within the 3700-3100 cm^{-1} region, which included absorbances attributed to O-H and N-H stretch. In addition, Pair 2 AMP soils had higher absorbance at 1560 cm^{-1} and 1540 cm^{-1} (Amide N-H), while Pair 5 AMP soils had slightly higher absorbance at 1650 cm^{-1} (Amide C=O) and 1720 cm^{-1} (Carboxylic acid C=O) compared to CG soils. Pairs 1, 4, and 5 showed greater clay peaks around 3700 cm^{-1} in AMP soils compared to CG. Pair 3 had a marked decrease in absorbance at 3700 cm^{-1} in AMP soils, suggesting that the higher soil C might be coating some of the clay material, preventing its detection.

4. Discussion

Consistent with our hypothesis, we observed that soils under AMP grazing had on average 9 Mg C ha^{-1} more soil organic C than soils under CG (Figure 1a; Supplemental Table 2). Our study sites had soil C stocks similar to those (i.e., 35-51 Mg C ha^{-1} down to 30-50cm depth) reported for grassland soils in the southeast region of the U.S., as well as other grassland regions of the U.S., Australia, and New Zealand (Machmuller et al., 2014; Hendrix et al., 1998; Conant et al., 2003; Stanley et al., 2019; Beare et al., 2014). It is however, difficult to compare across studies because of the diversity of grazing management types analyzed, the time since management conversion, and the unknown legacy of previous land uses. Despite these limitations, farms that implemented forms of rotational grazing in the southern U.S. had higher soil C stocks compared to other conventional forms of grazing (Conant et al., 2003; Teague et al., 2011). In other regions such as Australia, no significant differences in soil C stocks between rotational and conventional grazing have so far been reported, likely due to difficulty capturing paddock heterogeneity, and confounding fertilizer application effects (Sanderman et al., 2015; Chan et al., 2010). While our study demonstrates that AMP management results in higher soil C stocks along the soil profile compared to conventional grazing, there was a discrepancy between our results and results analyzing other types of rotational grazing from around the world. This points to the need for more world-wide testing of AMP grazing management effects on soil C stocks using comparable methodology and also an analysis of the drivers of soil C stock changes to enable generalization and forecasting of AMP management effects.

A first potential driver for the increases in soil C stocks is the increase in soil C inputs. While our study was not designed to quantify C inputs at the different farms, we measured standing roots and quantified light POM, which generally tracks structural C inputs (Christensen, 2001). We found large differences in root C stocks between farm pairs (Figure 2b; Supplemental Table 2). Contrary to our expectation, two out of the five CG farms had much greater root C stocks than AMP farms, while there was no difference between grazing types and root C stocks at the other three farms. Unfortunately, we only sampled roots once, and thus cannot make any inference on their productivity or turnover. However, we did not find any differences between grazing management types for light POM stocks (Figure 3; Supplemental Table 2). This SOM fraction is useful for tracking structural plant inputs because light POM represents plant litter inputs often in the early stages of decomposition (Christensen, 2001). This observation, coupled with the inconsistency between root C and soil C stocks suggest that either root structural inputs are not the primary source for soil C formation in these soils, or that root turnover is slower and does not necessarily result in efficient SOM formation in CG soils, as compared to AMP soils.

A second potential driver for increases in soil C stocks is changes in the quality of soil C inputs. Our isotopic results showed that there were differences in the plant community composition, which would affect the chemical quality of the plant inputs to the soil. Overall AMP farms consistently had lower natural abundance soil $\delta^{13}C$ signatures than CG farms (Figure 4a; Supplemental Table 4). The differences in soil $\delta^{13}C$ values between grazing managements are likely due to the photosynthetic pathways (i.e., C_3 vs. C_4) of the dominant vegetation, and/or its water use efficiency. It is possible that CG farms had more C_4 vegetation compared to AMP farms because these plants have a much higher $\delta^{13}C$ signature than C_3 plants (Farquhar et al., 1989). Lower soil $\delta^{13}C$ values can also indicate lower plant water stress and greater water use efficiency when there is similar aboveground vegetation (Farquhar et al., 1982). Thus, the overall lower soil $\delta^{13}C$ values on AMP farms could be due to higher abundance of C_3 and/or lower water stress. AMP farmers in Pairs 4 and 5 also indicated that they seeded cool season C_3 grasses. Carbon derived from C_3 plants has been found to have higher persistence in soils than from C_4 plants (Wynn and Bird, 2007). However, there was not a consistent relationship between the soil $\delta^{13}C$ and C stocks, indicating that vegetation type (C_3 vs. C_4) was not a dominant driver of soil C stock changes between grazing managements. On the other hand, the light and heavy POM fractions had lower C:N ratios under AMP grazing (Supplemental Table 3). The lower C:N ratio of the POM fractions on AMP farms may indicate higher quality inputs that are more accessible to microbes, which could lead to faster turnover of roots and SOM inputs as well as higher efficiencies in the utilization by microbes (Averill and Waring, 2018; Schimel and Weintraub, 2003), and therefore result in more efficient SOM formation, in particular of the MAOM fraction (Cotrufo et al., 2013).

Consistent with soil C stocks and the lower C:N of POM, we found that AMP had higher soil N stocks than CG farms across the sampled region (Figure 1b; Supplemental Table 2). On average, AMP grazing farms had soil N stocks that were 1 Mg N ha^{-1} higher than to CG farms. However, none of our AMP farms added inorganic N, whereas two of our CG farms (Pairs 2 and 3) implemented inorganic N inputs (Table 2). AMP farms have cattle in greater concentrations for shorter periods of time, which more evenly distributes organic N inputs from feces and urine to the soil without overloading it (Teague et al., 2018). Our findings confirm previous estimates of higher soil N stocks under rotational grazing compared to conventional grazing (Conant et al., 2003). However, other studies have found no differences in soil N stocks between grazing managements (Dubeux et al., 2006; Silveira et al., 2013; Altesor et al., 2006). This could be due to the fact that these studies were comparing grazing management practices that are different from the practices used in our study. Additionally, the discrepancy of the results could also be because these studies only compared grazed vs. un-grazed plots (Altesor et al., 2006), or N stocks were compared across a gradient of N fertilization rates (Dubeux et al., 2006) and only short-term responses were measured (Silveira et al., 2013).

Our isotopic results showed that AMP farms had lower natural abundance soil $\delta^{15}N$ than CG farms (Figure 4b). The differences in soil $\delta^{15}N$ between grazing managements could in part be due to inorganic N fertilization on CG farms from Pairs 2 and 3 (Table 2; Supplementary Table 2). Inorganic N fertilizers tend to have a higher $\delta^{15}N$ signature, which can increase the soil $\delta^{15}N$ values (Handley and Scrimgeour, 1997). However, we do not know the $\delta^{15}N$ natural abundance of these added inputs and cannot confirm that the increase in soil $\delta^{15}N$ on CG farms is a direct result of inorganic N fertilization. Soil N isotopes can also inform about the openness of the N cycle. Lower soil $\delta^{15}N$ values can indicate more efficient and less leaky N cycling (Handley and Scrimgeour, 1997). Our findings of higher N stocks in the farms that did not apply inorganic N fertilizers combined with the lower soil $\delta^{15}N$ signatures in particular at depth on AMP farms point to these farms being more efficient at cycling and retaining N in their soils.

As we hypothesized, AMP management also resulted in greater stabilization of the soil C stocks. We found higher MAOM C stocks in the soils under AMP grazing compared to the soil under CG (Figure 3; Supplemental Table 2). Based on the different pathways of MAOM and POM formation (Cotrufo et al., 2015; Haddix et al., 2016) we know that higher quality inputs and higher N availability can lead to higher microbial C use efficiency and increases in MAOM stocks (Cotrufo et al., 2013; Averill and Waring, 2018). This is because microbes need N for metabolism and previous research shows that the majority of MAOM has undergone some sort of microbial transformation (Miltner et al., 2002; Kallenbach et al., 2016). This further highlights the importance of N for microbial activity, their efficient transformation of C inputs, and MAOM formation. Greater soil N stocks as well as lowered C:N ratios of the POM fractions are likely the reason for why more SOM, specifically MAOM, was able for form and persist. Other studies have found mixed results when comparing MAOM fractions across grazing studies. For example, studies that found higher soil N stocks also reported higher MAOM stocks, whereas studies that showed no differences in soil N stocks also found no differences in MAOM stocks (Conant et al., 2003; Dubeux et al., 2006; Silveira et al., 2013; Altesor et al., 2006), confirming the high N demand of C sequestration in MAOM (Cotrufo et al., 2019; van Groenigen et al., 2006).

Similar to MAOM, we consistently saw higher heavy POM C stocks in soils under AMP grazing relative to soils under CG management (Figure 3; Supplemental Table 2). This sand-sized organic matter fraction is thought to be at the intermediate stages of decomposition and being contributed to by both plant and microbial products, but are not stabilized by strong mineral associations to silt and clay minerals (Christensen, 2001; Grandy and Neff, 2008). Other studies have found mixed results when comparing POM fractions across grazing studies which were influenced by things like different vegetation communities and fertilization gradients (Conant et al., 2003; Dubeux et al., 2006; Altesor et al., 2006). However, of the studies, none had identical grazing management comparisons or identical soil fraction schemes to the one we used here, which can make direct comparisons challenging. For example, SOM was only separated into POM and MAOM (Conant et al., 2003; Altesor et al., 2006) or into light and heavy SOM (Dubeux et al., 2006).

Our MID-IR data confirms that the soils from each farm pair are analogous (Supplemental Figure 1), pointing to any chemical differences being from differences in grazing management, not from soil type. Our findings from MID-IR scanning show increases in the mineral signal range for AMP farms. These higher mineral peaks could be due to textural differences (i.e., more clay); however, they may also be due to the increase in MAOM found on AMP farms, especially because MAOM was such a large proportion of the total SOM across our farms (Figure 5). Bands near the 3620-3700 cm^{-1} represent clay –OH absorbance in soils (Guillou et al., 2015), and it is possible that MAOM, due to its clay-rich nature, imparts soil with higher absorbance in this region. Overall, the chemical differences between grazing managements were very small. In some of the AMP farms we saw small increases in our fingerprint region which contains peak signals for organic matter components (Parikh et al., 2015). However, these differences were not consistent across farm pairs. Within this variability, AMP farms resulted in changes in organic matter moieties such as C-rich aliphatics to N-rich amides, which agree with our suggestions that the higher C stocks in AMP soils are due to more efficient microbial transformation of plant and animal inputs, rather than by increases in structural plant inputs.

5. Conclusions

Our findings show that the AMP grazing sites had on average 13% more soil C and 9% more soil N compared to the CG sites, across a 1 meter depth. The greater soil C stocks appears to be driven by the quality, and likely temporal and spatial distribution, of the C and N inputs and not so much by the quantity of structural plant inputs (i.e., roots and light POM). We found evidence for differences in plant community inputs based on our natural abundance $\delta^{13}C$ values. Additionally, SOM fractions available for microbial transformation were of higher quality, with lower C:N ratios on AMP grazing farms relative to CG farms. Since there can be no long-term C sequestration without available N, higher soil N stocks and N retention in addition to lower C:N ratios in the POM fractions lead to significantly more persistent C in the MAOM fraction on AMP grazing farms compared to CG farms. Overall, on average AMP farms had higher soil C and N stocks, lower soil $\delta^{15}N$ signatures, as well as lower C:N ratios in the majority of SOM fractions relative to CG farms, which highlights the potential of AMP farms to retain more N and sequester more C. These findings provide evidence that AMP grazing management could be implemented at large scales as a way to sequester persistent C and mitigate rising atmospheric CO_2 levels.

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Table 1. Farm pair locations, climate information, soil series and slope descriptions, soil taxa according to USDA taxonomy, as well as individual farm average A-horizon depths and A-horizon soil textures separated by adaptive multi-paddock (AMP) and conventional grazed (CG) farms.

Farm Pair	Farm Location	MAT (°C)	MAP (cm)	Grazing Practice	Slope (%)	Soil Series	Soil Taxonomy	Average A-horizon depth ± standard errors (cm)	Average A-horizon textures %sand, %silt, % clay (± standard errors)
1	Adolphus, Kentucky	13.75	131.57	AMP	2-6	Trimble gravelly silt loam	Fine-loamy, siliceous, semiactive, mesic Paleudults	13.87 ± 0.56	16.76 (1.58), 52.58 (2.14), 30.66 (0.82)
					6-12				
				CG	2-6			13.51 ± 0.44	25.87 (1.15), 49.64 (1.66), 24.49 (1.11)
					6-12				
2	Sequatchie, Tennessee	14.17	143.15	AMP	0-2	Emory sil loam	Fine-loamy, siliceous, semiactive, Typic Paleudults	14.40 ± 0.80	30.37 (1.60), 35.86 (3.10), 33.76 (2.77)
					2-5	Cumerland silty clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudalfs		
				CG	0-2	Emory sil loam	Fine-loamy, siliceous, semiactive, Typic Paleudults	11.93 ± 0.46	43.11 (1.95), 28.27 (1.46), 28.62 (2.74)
					2-5	Cumerland silty clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudalfs		
3	Fort Payne, Alabama	15.11	141.96	AMP	2-6	Hartsell fine sandy loam	Fine-loamy, siliceous, semiactive, Typic Hapludults	13.33 ± 0.39	64.36 (2.89), 20.95 (1.85), 14.69 (1.22)
					6-10				
				CG	2-6			12.11 ± 0.41	70.04 (1.05), 14.95 (0.78), 15.01 (0.94)
					6-10				
4	Piedmont, Alabama	15.67	135.23	AMP	2-6	Cumberland gravelly loam	Fine, kaolinitic, thermic Rhodic Paleudults	11.65 ± 0.46	47.29 (1.74), 25.99 (2.83), 26.72 (1.88)
					6-10	Cumberland gravelly clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudalfs		
				CG	2-6	Cumberland gravelly loam	Fine, kaolinitic, thermic Rhodic Paleudults	12.04 ± 0.4	54.81 (2.92), 24.62 (1.76), 20.57 (1.52)
					6-10	Cumberland gravelly clay loam	Fine, mixed, semiactive, thermic Rhodic Paleudalfs		
5	Woodville, Mississippi	19.00	164.87	AMP	2-5	Loring silt loam	Fine-silty, mixed, active, thermic Oxyaquic Fagiudalfs	9.87 ± 0.50	23.75 (1.24), 56.63 (1.73), 19.62 (1.01)
					5-8				
				CG	2-5			9.16 ± 0.42	18.38 (2.88), 64.31 (2.64), 17.30 (0.55)
					5-8				

Table 2. Average farm management information from 2018 for each adaptive multi-paddock (AMP) and conventional grazed (CG) farm pair.

Farm Pair	Grazing Practice	Livestock in study area	Total grazeable land (ha)	Total # of animal units (AU)	Average stocking rate (AU/ha)	# of herds	# of AU per herd	Average # of paddocks	Average paddock size (ha)	Average stocking density (AU/ha)	Average grazing period goal (days)	Time to cover full farm (days)	Rest vs. grazed period ratio	Inorganic N inputs	Other inputs	Herbicide inputs	Lime inputs	Length of current mangement (years)	Land use history
1	AMP	beef cattle, sheep	83	115	1.39	1	115	45	1.84	62.35	2	90	44.00	none	none	none	1.5 tons/acre (0.14x/year)	13	Tobacco & grain crops
	CG	beef cattle	14	11	0.79	1	11	1	14.00	0.79	365	365	0.00	none	none	none	none	6	Tobacco & grain crops
2	AMP	beef cattle	44	113	2.57	1	113	45	0.98	115.57	2	90	44.00	none	none	none	none	12	Row cropped, hay, and grazing
	CG	beef cattle	122	82	0.67	3	27	8	15.25	1.79	135	360	1.67	125 lbs/acre (1x/year) Triple 19	none	1.25 gal/acre (1x/year) 2,4 D	none	>25	Row cropped, hay, and grazing
3	AMP	beef cattle	100	155	1.55	1	155	60	1.67	93.00	1	60	59.00	none	2.5 gal/acre (1x/year) fish emulsion and 5 lbs/acre (1x/year) sea-90 salt	none	none	29	Small grains
	CG	beef cattle	850	700	0.82	14	50	30	28.33	1.76	365	782	1.14	300 lbs/acre (1x/year) Commercial N	1.5 tons/acre (1x/year) Chicken litter	none	1 ton/acre (0.33x/yr)	17	Small grains
4	AMP	beef cattle	37	140	3.78	1	140	123	0.30	465.41	1	123	122.00	none	none	none	none	24	Cotton
	CG	beef cattle	36	35	0.97	1	35	2	18.00	1.94	365	730	1.00	none	none	none	none	>40	Cotton
5	AMP	beef cattle	216	225	1.04	1	225	150	1.44	156.25	1	150	149.00	none	none	none	none	10	Tobacco & grain crops
	CG	beef cattle	65	71	1.09	1	71	7	9.29	7.65	75	525	6.00	none	none	none	none	38	Tobacoo, cotton, market gardening & grain crops

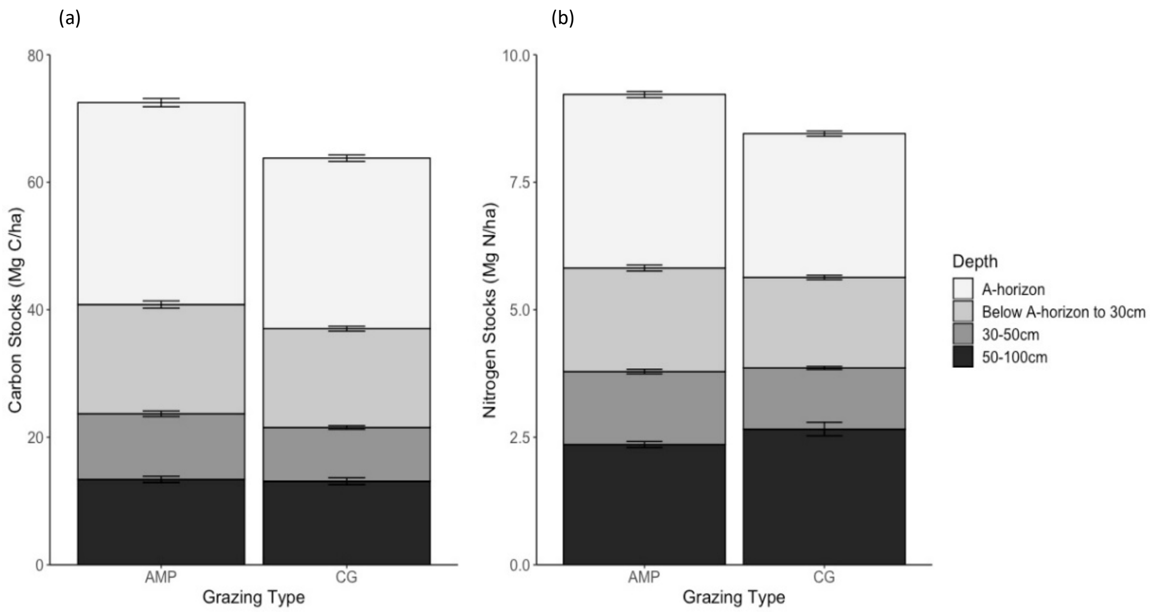

Figure 1. Total soil organic C stocks (a) and total soil N stocks (b) down to 1 meter separated by depth increment and by adaptive multi-paddock (AMP) and conventional grazing (CG) managements. Error bars represent standard errors (n=377 for each depth increment).

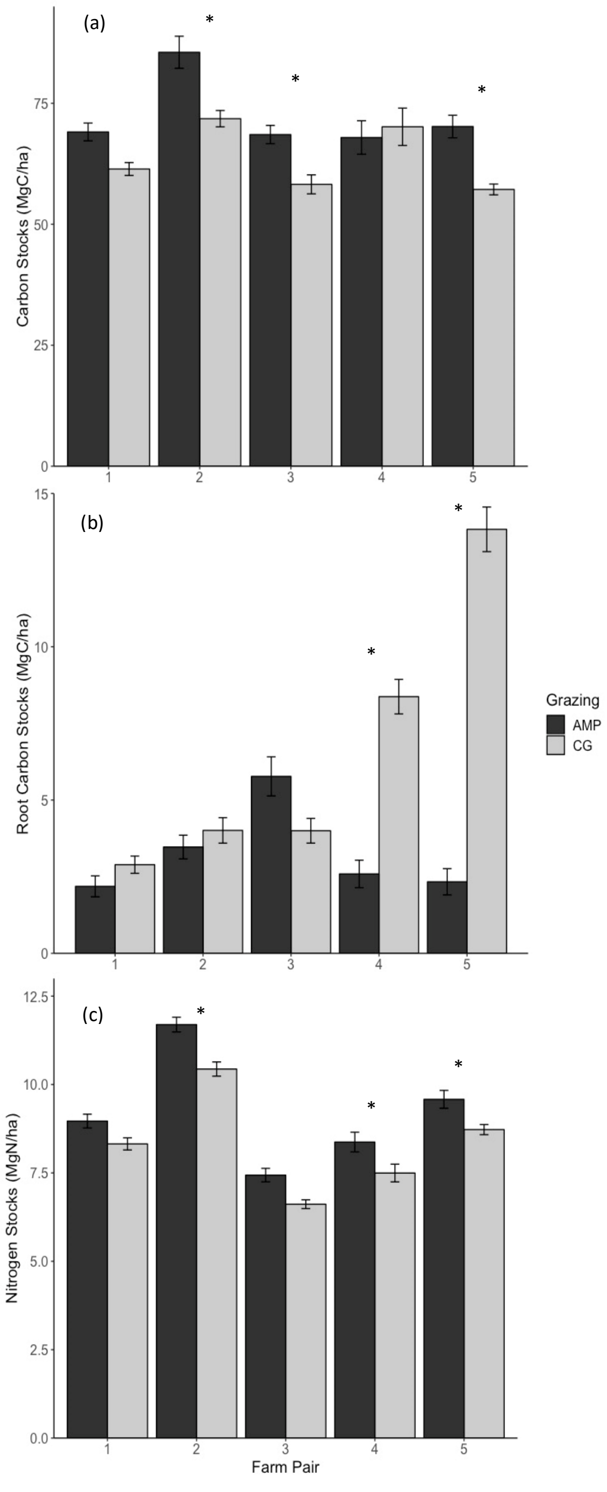

Figure 2. Total organic C stocks (a), total root biomass C stocks (b), and total soil N stocks (c) down to 1 meter separated by farm pairs and by adaptive multi-paddock (AMP) and continuous (CG) grazing managements. Data are averages (n=30-42 per farm), with error bars representing standard errors. Asterisks denote significant differences (p-value<0.05) between farm pairs.

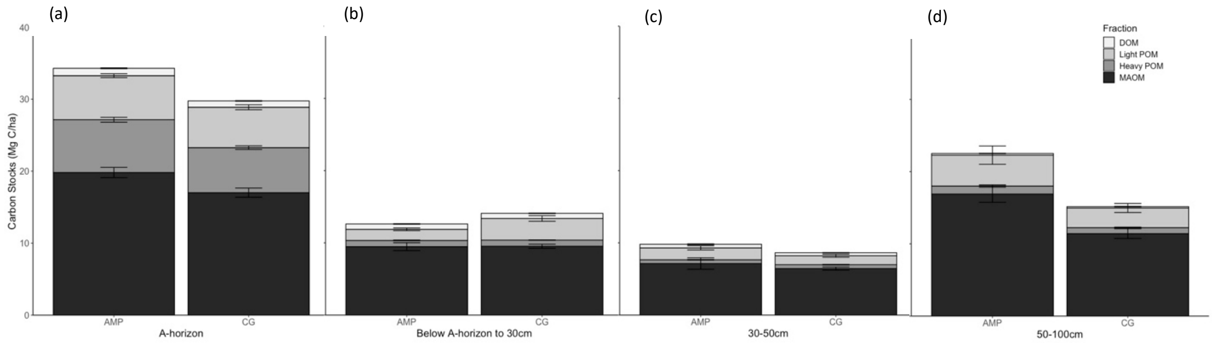

Figure 3. Soil C stocks separated by soil organic matter fraction distribution for A-horizon (a), below A-horizon to 30cm (b), 30-50cm (c), and 50-100cm (d) depth increments, for the adaptive multi-paddock (AMP) and continuous (CG) grazing managements. Data are averages (n=54-60 for each fraction and depth), with error bars representing standard errors. DOM is dissolved organic matter, POM is particulate organic matter, and MAOM is mineral associated organic matter.

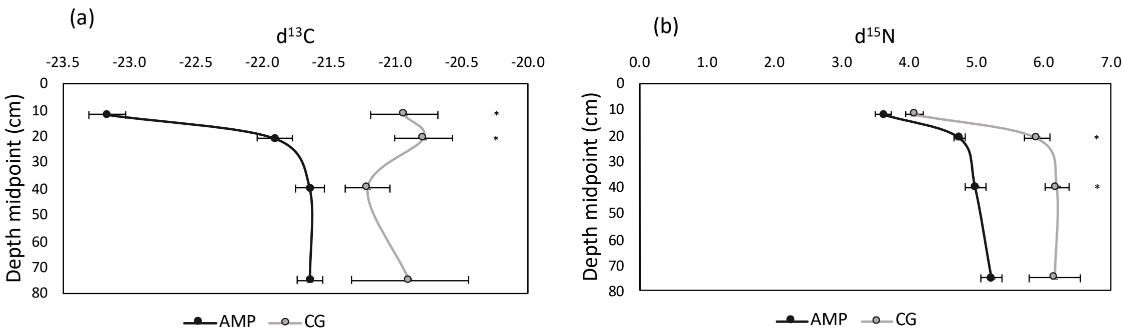

Figure 4. Natural abundance isotopic values for (a) bulk soil $\delta^{13}C$ and (b) bulk soil $\delta^{15}N$ in adaptive multi-paddock (AMP) and continuous (CG) grazing grasslands across each depth increment midpoint (cm). Asterisks denote significant differences ($p<0.05$) between grazing types at each depth increment. Data are averages (n=394-420) with error bars representing standard errors.

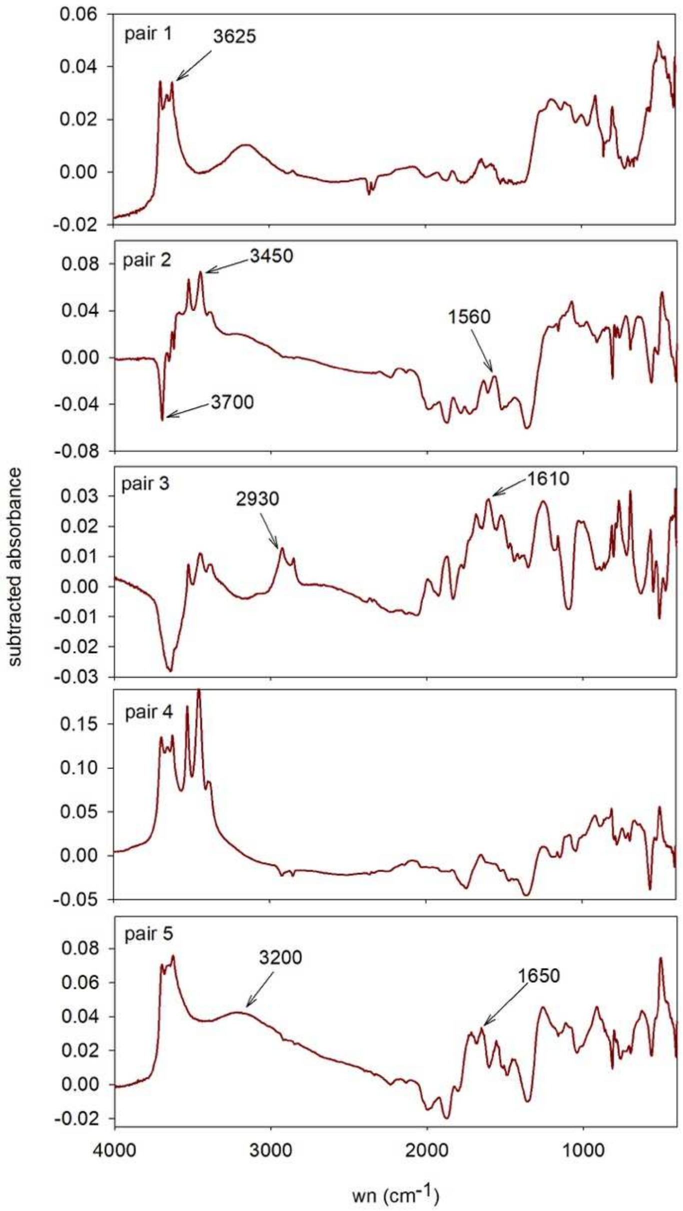

Figure 5. Subtractions of the Fourier transformed mid infrared diffuse reflectance spectra of the A-horizon soils between adaptive multi-paddock (AMP) and continuous (CG) grazing treatments for each farm pair (n=42 per farm). This data represents the baseline corrected AMP spectral average minus the CG average spectrum.