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Soil health in an organic, integrated crop-livestock system in California:
a close look at soil C and N dynamics

By

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THESIS

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1 Abstract

Grazing cover crops provides an opportunity to reconnect crop and livestock production on the same land with potential to enhance soil health. However, critical knowledge gaps currently hamper adoption of these diversified systems in organic vegetable production. There is a need to better understand shifts in soil health with grazing of cover crops, especially impacts on soil carbon (C) and nitrogen (N) pools, and the chemical, biological and physical properties of soil regulating sustainable organic vegetable production. We investigated how sheep integration impacts major indicators of soil health of relevance to growers, including soil physical (compaction and soil structure), chemical (pH, salts, macronutrients, labile C and N pools and soil organic matter fractions), and biological (microbial composition and function) properties. We conducted a 4-year randomized complete block design experiment with three winter cover treatments: fallow, ungrazed cover crop and grazed cover crop, in an organic vegetable crop rotation. We found that grazing did not significantly impact soil physical characteristics. Cover crop establishment and grazing did not alter dissolved organic C pools, while cover crops and grazing did lower soil pH. Nitrogen was the only soil nutrient impacted by cover crops or grazing. At moments key to crop production, grazing cover crops increased N availability in organic, inorganic, and microbial pools beyond what ungrazed cover crops could provide. Grazing did not lead to increased potentially leachable nitrate despite greater inorganic N pools during the cropping season. Grazing decreased the fungal/bacteria ratio and increased the gram (+) / gram (-) bacterial ratio, reflecting the increase in labile nutrients. Shifts in the timing of nutrient release and carbon flows with grazing did not lead to significant changes in mineral associated organic matter (MAOM) though there was a trend towards lower POM with 4 years of winter grazing, which needs future longer-term assessment. Organic farmers have the potential to utilize sheep grazing to strategically improve the timing of N release for their crops, with minimal tradeoffs in terms of physical properties, when grazing implementation follows best management practices.

2 Introduction

Large-scale specialization and globalization of our food system has led to the separation of crop and livestock production, particularly in industrialized countries. Crops for human consumption or animal feed are mostly produced on prime farmland and animals are raised in confined operations or grazed on more extensive rangelands (Byrnes et al., 2018; Liu et al., 2017; Wang et al., 2016; Zhou et al., 2017). Due to this separation, nitrogen (N) and carbon (C) inputs from animal urine and manure are underutilized to support crop growth, which disconnects nutrient cycles across temporal and spatial scales. Specialization of crop systems can generate imbalances in nutrient cycling, leading to losses of nutrients and soil organic matter (SOM) which contribute to poor soil health and environmental degradation (Watson et al., 2019). Likewise, specialization of intensive livestock enterprises creates nutrient excesses leading to storage and pollution challenges (Goyette et al., 2023) while extensive systems may encroach on native grasslands or not be able to support livestock production throughout the seasons (de Faccio Carvalho et al., 2021).

Integrated crop-livestock systems (ICLS), in which animals are produced on the same land, simultaneously or in the same rotation, as crops, offer an opportunity to reconnect landscape-scale nutrient cycles by linking waste from animals with crop nutrient demands and provide *in situ* forage for animals. Animals in ICLS consume unharvested plant biomass, such as cover crops, weeds, crop stubble or even crops themselves in their early stage (known as a dual-purpose crops) (Garrett et al., 2017). While most ICLS are found in smallholder systems, examples of ICLS in large-scale, industrial agriculture include beef cattle grazing in cereal and soybean systems in the United States (Franzluebbers & Stuedemann, 2008b) and Brazil (F. D. da Silva, Amado, Ferreira, et al., 2014) and silvopastoral systems such as sheep grazing in vineyards in New Zealand and the US (Garrett et al., 2017; Ryschawy et al., 2021). Globally, ICLS can provide greater food security (Sekaran, Lai, et al., 2021) without impacting crop yields compared to unintegrated systems, except in dual-purpose crops where yield lags may occur (Peterson et al., 2020). ICLS could therefore help realize major tenets of sustainable crop and animal production (Van Zanten et al., 2018) with animals consuming crops that humans cannot and the integration of animals lowering the

risk of nutrient excesses coming from intensive operations and decreasing further expansion of agriculture into other landscapes.

Livestock integration profoundly impacts soil health and ecosystems and provides an opportunity to promote recycling and conservation of C and N within a system (J. M. Assmann et al., 2015; F. D. da Silva, Amado, Bredemeier, et al., 2014; Franzluebbers & Stuedemann, 2015). Studies examining the effect of ICLS on net primary productivity (Allan et al., 2016; McKenzie et al., 2016a; Peterson et al., 2020), decomposition (T. S. Assmann et al., 2014), and soil C and N pools have shown that a moderate grazing intensity can lead to C and N storage (Abdalla et al., 2018; F. D. da Silva, Amado, Bredemeier, et al., 2014; Medina-Roldán et al., 2008), and greater availability of labile C and N for crop production (J. M. Assmann et al., 2015; Tian et al., 2010). However, these impacts are system dependent and grazing influences the factors that control soil organic carbon (SOC) and soil organic nitrogen (SON) pools in a complex way (Piñeiro et al., 2010).

Outcomes of livestock grazing of winter cover crops (CC) on soil health are context specific and haven't been thoroughly explored. Thus, the state of knowledge is low in organic, tilled vegetable systems. Organic farmers need to better understand how grazing CC alters soil health indicators compared to both a winter fallow field and an ungrazed CC field because they rely heavily on internal nutrient cycling and require nuanced temporal cycling information to adapt their management and minimize potential tradeoffs. Organic agriculture is, therefore, an ideal system to implement ICLS and better use ecosystem services provided by animals for crop production and to evaluate outcomes on soil health.

Animal integration can impact soil physical properties through trampling and hoof action (Figure 1A). High intensity grazing is associated with increased compaction (Lai & Kumar, 2020), while research in grain, no-till ICLS measured no biologically detrimental increase in bulk density (a metric of compaction) with grazing at low intensities (H. A. da Silva et al., 2014). Grazing in an integrated market garden decreased compaction compared to mowing (McKenzie et al., 2016b) but it is unclear how grazing impacts compaction in tilled, industrial, vegetable systems. Aggregate stability (a metric of soil structure

and proxy for erodibility) has been shown to increase or remain unchanged with grazing (Figure 1A) (Acosta-Martínez et al., 2004; Brewer & Gaudin, 2020; Franzluebbers & Stuedemann, 2008b).

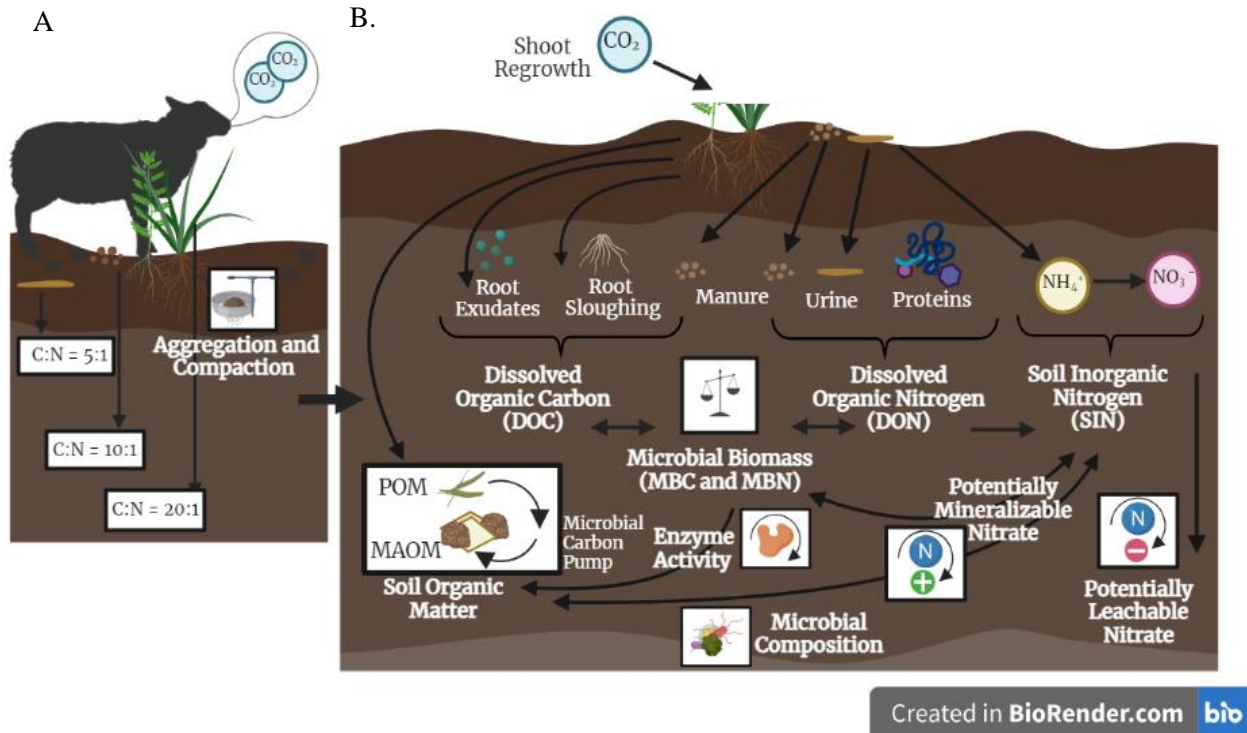


Figure 1. Potential mechanisms associated with soil health outcomes in grazed systems.

Panel A - Compaction and aggregation could increase due to animal integration. Grazing converts plant biomass (C/N ~ 20:1) to manure and urine which have a lower C/N ratio (~10:1 and 5:1, respectively) and impact downstream soil chemical and biological properties. **Panel B** - As a result of the mineralization of lower C/N ratio inputs, and of plant responses to grazing (regrowth, root exudates, root sloughing), a grazed system may have greater DOC and DON which contribute to the MBC and MBN pools. Labile nitrogen (SIN) may also be greater, which could contribute to plant uptake, microbial pools, or be lost through leaching. Microbial composition and enzyme activity could be altered by grazing, impacting the transformation of POM into MAOM through the microbial carbon pump.

At its core, grazing transforms plant residues into urine and manure which impacts chemical soil properties with downstream effects on soil biological properties. For instance, pH, a regulator of soil health and nutrient availability, can increase with grazing as labile N as urea in urine is rapidly hydrolyzed to NH_4 (Williams & Haynes, 1994). However, complete nitrification of urea (which takes 14-29 days after application (Haynes & Williams, 1993; Williams & Haynes, 1994)) leads to acidification (Liebig et al., 2016), and one study found subsoil acidification from applying synthetic urine (Condon et al., 2020).

Grazing could alter the timing and loss potential of soil inorganic N (SIN) released from manure and urine compared to plant biomass. Plants (\sim C/N = 20:1) are transformed into manure and urine, which have a lower C/N (\sim 10:1 and 5:1, respectively) due to ruminant digestion and CO₂ losses via respiration for metabolic maintenance (Figure 1A) (Soussana & Lemaire, 2014). Carbon to nitrogen ratios of inputs correlate negatively with N mineralized over a few months (Lazicki et al., 2020). Thus, nutrients from manure and urine may mineralize quicker than that of ungrazed plant biomass and make N more temporally plant bioavailable in grazed systems (Grandy et al., 2022). Manure has a consistent N content of 8 g N kg⁻¹ dry matter consumed (Haynes & Williams, 1993), with a majority of manure N being in an organic form (Sørensen & Jensen, 1995). Because manure supports soil microbial heterotrophy, it can contribute to the storage of N into SOM with a low loss potential. One study found that cattle manure, when mixed with grassland soil in controlled conditions, increased soil microbial biomass carbon (MBC) and nitrogen (MBN), though these effects could be diluted in field conditions (Lovell & Jarvis, 1996). On the other hand, urine could potentially be a source of N loss because N excreted in urine is predominantly inorganic. The amount of N in urine varies based on the N content of the forage consumed, with greater forage N leading to higher N in urine (Haynes & Williams, 1993). Up to 50% of excreted N in urine is urea, which is rapidly hydrolyzed to NH₄ and then nitrified more slowly (Haynes & Williams, 1993; Williams & Haynes, 1994). It is unclear how NO₃⁻ leaching is changed with grazing in ICLS (Garrett et al., 2017), though studies in extensive range systems have shown that grazing could lead to N immobilization in microbial biomass allowing for synchronous release of N with plant demand and no additional NO₃⁻ leaching concerns (Frank et al., 2000; Hoogendoorn et al., 2016). In contrast, one study found significantly higher NO₃⁻-N below the rooting zone after 3 and 4 years of livestock integration (Franzluebbers & Stuedemann, 2013). However, NO₃⁻ leaching was not measured so impacts on this loss pathway remain unclear. Additionally, high amounts of soil NO₃⁻, in conjunction with soil C, can lead to denitrification and N₂O emissions, which could be increased with grazing (Lazcano et al., 2022), though this is not evaluated in our study.

Plant responses to grazing, both above and below ground, can also lead to alterations in the amount and quality of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), and the size of the SIN pool (Figure 1B). Quantity of aboveground biomass production is closely correlated with total C and N release from residues (J. M. Assmann et al., 2015) and although grazing could lead to lower aboveground biomass inputs (Dai et al., 2019), shifts belowground may mitigate these impacts. Root litter (including exudates and sloughed off dead roots) contribute significantly to plant biomass inputs into the soil, yet are often overlooked due to challenges associated with root quantification (Smith et al., 2014). Grazing can stimulate root biomass (Dai et al., 2019) and turnover (Roberts & Johnson, 2021; Zhang et al., 2022), impacting the quantity, quality and timing of C and N inputs. Grazing can also enhance plant exudation, particularly in grass species, for about 3-5 days post grazing (Hamilton et al., 2008; Holland et al., 1996; Paterson & Sim, 2000) which has been shown to rapidly double rhizosphere soil C and microbial C content in the time immediately after the grazing event (Hamilton & Frank, 2001) and contribute to higher concentrations of bulk soil DOC (Tian et al., 2010) and could stimulate microbial growth leading to higher microbial biomass in grazed systems (Zhou et al., 2017). Grazing keeps plants at earlier developmental stages, when root exudation is greater, thus also potentially contributing to greater DOC inputs over the season (Six et al., 2004). Nitrogen is also released from roots but at a lower quantity relative to C, leading to a higher C/N ratio of exudates which has been associated with microbial release of N from other SOM pools in extensively grazed systems (Hamilton & Frank, 2001).

On arable land with greater N cycling, net impacts of N inputs from roots, aboveground biomass, manure, and urine may differ from rangeland systems. When the direct and indirect impacts of grazing on the N cycle are taken together, grazing has the potential to improve the timing of nutrient release, such that there is a higher proportion of N in organic vs inorganic forms at crop planting and the inverse during the crop growth, compared to ungrazed systems. This is similar to a tightly coupled N cycle, which has a high proportion of N in SOM and less in inorganic forms leading to low potential for N loss, yet adequate N availability during the growing season (Bowles et al., 2015).

It is difficult to predict the net effect of labile N on organic pools and ratios of C and N because labile N could contribute to both DOC and DON formation and consumption processes simultaneously (Filep & Rékási, 2011). In one study, increasing labile N was correlated with increased DOC concentrations (Filep & Rékási, 2011), whereas in another study a lower soil DOC/DON ratio reflected a nutrient rich system (Cookson et al., 2007). This ratio hasn't been evaluated extensively in grazed systems, but the ratio could verify other soil chemical and biological analyses to understand how grazing impacts soil nutrient status and labile soil organic pools.

Grazing also has direct and indirect impacts on soil microbial community composition and functioning which are challenging to predict (Tian et al., 2010). Sheep consumption of plant biomass decreases the total amount of cellulose and lignin entering the soil, and thus, could contribute to a decrease in both the activity of β -Glucosidase (catalyzes degradation of cellulose) and abundance of fungi. In general, arbuscular mycorrhizae (AM) fungi abundance is low in high nutrient conditions, such as crop production, and tillage disrupts their hyphae. Additionally, defoliation reduces colonization rates of AM fungi (Ilmarinen et al., 2008) and, thus, grazing may further lower AM fungi populations. In one organic ICLS study, an increase in soluble phenolics was correlated with a decrease in β -Glucosidase activity while β -Glucosaminidase (catalyzes the degradation of chitin) was found to be greater with grazing than without grazing (Tian et al., 2010). Finally, gram (+) bacteria can decompose complex POM and recalcitrant substrates, when N availability is high (Orwin et al., 2018) and proliferate with disturbance, while gram (-) bacteria are associated with low disturbance (Klumpp et al., 2009; Lange et al., 2019), thus grazing may increase the gram (+) / gram (-) ratio.

Alterations to the chemical, biological and physical properties of soils with grazing may ultimately impact SOM and C storage pools both on the short and longer terms. Grazing provides a pulse of bioavailable C and N which once assimilated by microbes into MBC and MBN, could contribute to the mineral associated organic matter (MAOM) fraction through the soil microbial carbon pump (MCP) (Figure 1B) (Zhu et al., 2020). In this theory, continuous and cyclical microbial growth and death creates necromass

that contributes to between 33-70% of total SOM, particularly in the MAOM fraction. MAOM is composed of smaller, simpler compounds (including necromass, exudates and leached plant compounds) which are more assimilable by plants and microbes, but are associated with silt and clay minerals smaller than 20-63 μm , and thus, protected from further decomposition (Lavallee et al., 2020). The plant roots and shoots contribute to the particulate organic matter (POM) fraction, which is made up of larger, insoluble molecules that require depolymerization prior to plant or microbial assimilation and are associated with the fraction of soil larger than 20-63 μm (Lavallee et al., 2020). One recent meta-analysis found that grazing of cover crops raises soil organic carbon and significantly shifts the distribution of carbon from POM to MAOM (Prairie et al., 2023). In these systems, bioavailable N additions from animals could be decreasing the N limitations to POM decomposition which could lead to greater microbial processing of POM into MAOM via microbial growth and death (Lavallee et al., 2020).

The goal of this study was to elucidate how sheep grazing of winter cover crops in tilled, organic, vegetable cropping systems alters soil physical, chemical, and biological properties of relevance to growers. We implemented a field experiment that compared 3 winter management practices over 4 years: a) ungrazed cover crop (ungrazed CC), b) cover crop grazed by sheep (grazed CC), and c) fallow. We expected 1) soil physical properties to not be altered by grazing, 2) soil N and P to increase in bioavailability and soil pH to decrease with grazing, and 3) soil microbial community composition and activity to reflect a more labile nutrient status in the grazed system. We also more closely evaluated how labile soil C and N pools were affected by grazing and downstream impacts on SOM pools, N losses and N retention potential. We hypothesized that grazing would lead to 1) greater concentrations of bioavailable C and N (DOC and DON) and 2) higher microbial biomass (MBC and MBN) which would 3) mitigate the risk of N loss via nitrate leaching and 4) drive the MCP to decrease C in the POM fraction and increase C in the MAOM fraction.

3 Site description

Our ICLS field trial was implemented at the Russell Ranch Sustainable Agriculture Facility at the University of California, Davis in northern California (USA). The site experiences a Mediterranean climate characterized by wet, cool winters, with an average December temperature of 2.9° C, and hot, dry summers, with an average July temperature of 33.7° C (Wolf et al., 2018). Average yearly precipitation is about 440 mm yr⁻¹ (Wolf et al., 2018) and the site received 731, 295, 133, 401 mm of precipitation in the 2018-2019, 2019-2020, 2020-2021, and 2021-2022 field years, respectively. The trial was located along the Putah Creek alluvial fan which has two soil types: Yolo silt loam (fine-silty, mixed nonacid, thermic, Typic Xerorthents) and Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralfs) (Wolf et al., 2018), though textural analysis showed our site has a clay loam/clay texture in the top 30 cm.

4 Experimental design

We implemented a randomized complete block design (RCBD) with four replicates that compared three winter management treatments: ungrazed cover crop (ungrazed CC), grazed cover crop by sheep (grazed CC) and fallow in 4-year vegetable rotation (Figure 2A). Our cover crops were seeded using a Tye drill in November of each year and were reliant on rainfall for germination and growth. Poultry manure compost was the only fertility added to the system and was applied at cover crop seeding in the last 3 years. In the spring, all beds were disced, listed, and planted in the summer crop. Tomatoes were transplanted, while maize and cucumber were direct seeded. All crops were irrigated using buried sub-surface drip tape.

The first year (2019), a cover crop mix (30% fava bean, 30% field pea, 20% vetch, 20% oats) was grazed once, no compost was applied, and maize was grown. The second and third year (2020 and 2021), a cover crop mix (16% annual ryegrass, 16% common vetch and 67% field pea) was grazed by sheep twice and processing tomatoes were grown and poultry manure compost (1.8N-3P-2K) was applied at rate of 4- and 8-ton acre⁻¹, in fall 2019 and 2020, respectively. The fourth year (2022), a cover crop mix (75% cereal

rye, 12.5% crimson clover and 12.5% daikon radish) was grazed once, poultry manure was applied at a rate of 8-ton acre⁻¹ and cucumbers were grown.

Sheep grazed for 8 days in Year 1 at a rate of about 15 sheep acre⁻¹ day⁻¹. Sheep grazed for a range of 1-3 days, in Years 2-4, depending on the amount of CC biomass available. In Year 2, the first grazing event had about 27 sheep acre⁻¹ day⁻¹ and the second had 40 sheep acre⁻¹ day⁻¹. In Year 3, the first grazing event had about 26 sheep acre⁻¹ day⁻¹ and the second had 30 sheep acre⁻¹ day⁻¹. In Year 4, there were 200 sheep acre⁻¹ day⁻¹ for the one grazing event. Grazing rates varied based on forage availability and we aimed for moderate grazing intensity, which is defined as post-graze sward height of about 20 cm (de Albuquerque Nunes et al., 2019), because higher intensity grazing (sward height of <10 cm) has been associated with SOM degradation (J. M. Assmann et al., 2014) and compaction. The sheep were a mixture of Dorset, Suffolk/Hampshire crosses, and Dorset/Southdown crosses that were 1-5 years old.

We present results from the second (2020) and third (2021) years in which we measured various soil physical, chemical, and biological properties and evaluated labile soil carbon and nitrogen pools closely, and from the fourth year (2022) in which a final assessment of carbon pools was conducted.

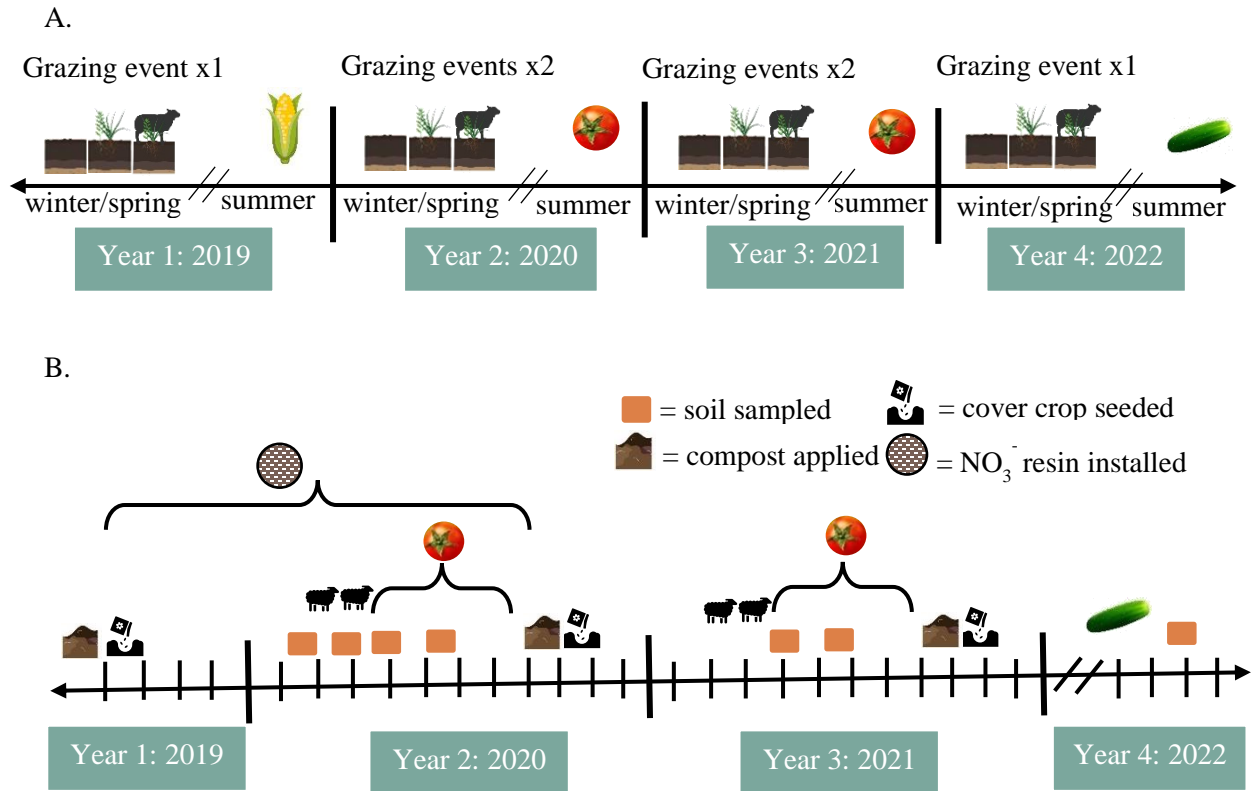


Figure 2. Experimental timeline and timing of field operations and soil sampling.

Panel A – Our trial included three winter treatments (fallow, ungrazed cover crop and grazed cover crop) in a 4-year rotation of maize-tomato-tomato-cucumber. **Panel B** – We applied compost and seeded the cover crop every fall. Nitrate resin bags were installed from 2019-2020. Grazing occurred every spring and in summer crops were grown. Soil sampling varied year to year.

5 Soil sampling

Two subsamples (taken ± 1 m apart from each other) were taken from a random location per plot using an auger and composited to form each sample. In Years 2 and 3, half the plots had 1 sample per plot and the other half had 2 samples per plot (which was taken into account by using plot as random effect in the models). In Year 4, only one sample was taken per plot. Soil was collected 30-40 cm from the center of the 3-foot beds. Bulk density samples (0-15 cm) were taken using a slide hammer at peak crop nutrient uptake. Soil was kept on ice until returned for processing.

Soil samples in Year 2 (taken at 0-15, 15-30 and 30-60 cm depth intervals) were collected and analyzed at: a) pregraze 1 (PreG1) b) pregraze 2 (PreG2) c) crop transplanting (CropT) and d) peak crop nutrient uptake (NutrientU) (Figure 2B). Soil samples in Year 3 (taken at 0-15 and 15-30 cm depth intervals) were collected and analyzed at: a) CropT and b) NutrientU. Soil samples in Year 4 (taken at 0-15, 15-30 and 30-60 cm depth intervals) were collected and analyzed at NutrientU. PreG1 and PreG2 soil was collected within 9 days before the grazing events in Year 2. CropT soil was collected within 2 days after the crop was transplanted or seeded in Years 2 and 3. NutrientU soil was collected within ± 1 week of the crop's peak nutrient demand in Years 2, 3 and 4.

6 Soil analyses

6.1 Soil physical properties

Bulk density was calculated from the mass of the dry soil core and the core volume. Gravimetric water content (GWC) was determined by drying soil at 60 °C until stable weights were obtained and was calculated by subtracting the oven-dried (OD) weight of soil from the fresh soil weight and dividing by the OD weight.

Soil wet aggregate stability was determined using an automatic soil sieve with rainfall simulator (Fritsch Analysette 3 Pro – Idar-Obstein, Germany) (Brewer et al., 2022). Sieves were set on the machine in the following order 1) 53 μm , 2) 250 μm 3) 2 mm, from bottom to top, with each soil sample placed on the 2 mm sieve. Briefly, 30 g of 8 mm sieved, then air-dried soil was sieved through the machine into 4 fractions >2 mm (large macroaggregates), 2-250 μm (small macroaggregates), 250-53 μm (microaggregates), and <53 μm (silt and clay fraction) with the amplitude set to 0.5 mm. First, the rainfall simulator was turned on for 15 s, then the shaker was turned on for 15 s in conjunction with rainfall simulator. After this, the >2 mm fraction was manually sprayed with a deionized water (DI) bottle, until it ran clear (~ 30 -60 s), and then the fraction was gently sprayed from the 2 mm sieve using DI into a metal loaf tin. Then, the rainfall simulator and shaker were turned on for 15 s simultaneously and the 2-250 μm

fraction was manually sprayed until it ran clear and was removed from atop the 250 μm sieve like the previous fraction. This process was repeated for the next fraction. The remaining soil in the machine was washed down the drainpipe into the $<53 \mu\text{m}$ loaf tin. The four fractions were dried at 60 $^{\circ}\text{C}$ for about 2 weeks, until all the moisture was evaporated, and the weights stabilized.

Mean weight diameter, a weighted average of aggregate stability, was calculated using equation 1 (van Bavel, 1950):

$$(1) \quad MWD = \sum_{i=1}^4 D_i \times P_i ,$$

where D_i is the average diameter of particles in each i -level aggregate fraction and P_i is the percent of that fraction in each sample.

6.2 Soil chemical properties

Fresh soil was sent to a commercial laboratory (Ward Laboratories – Kearney, NE) for pH (1:1 v/v method), electrical conductivity (EC, mmho cm^{-1}), available P (Olsen bicarbonate extraction, mg kg^{-1}), potassium, calcium, magnesium and sodium (ammonium acetate extraction, mg kg^{-1}) and cation exchange capacity ($\text{Meq } 100\text{g}^{-1}$) analyses.

6.3 Soil inorganic nitrogen pools and processes

Soil inorganic N (SIN) was extracted from field moist soil with 2 M KCl and analyzed for ammonium (NH_4^+) and NO_3^- (Doane & Horwath, 2003; Miranda et al., 2001). Potentially mineralizable nitrogen (PMN) was only evaluated at CropT in Year 2 and was measured using anaerobic incubation for 7 days (Drinkwater et al., 1996).

Nitrate leaching was evaluated in the second year (2019-2020, Figure 2B) using subterranean anion exchange resin bead (AMBERLITE™ PWA5 Resin) “pucks” placed at 65 cm for 11 months, from October 2019 to September 2020. Resin bead pucks were built following the methods in Woodward et al. (2022) protocol and described in Supp Fig 1. Each plot had 6 pucks installed under an undisturbed soil column

within 5 feet of each other. Nitrate was extracted from the resin beads by taking a subsample of 6 g, adding 30 ml of 2M KCl and shaking on a reciprocal shaker for one hour. Beads were allowed to settle, and the supernatant was decanted. Two more rounds of extractions using 2M KCl were conducted. These three extracts were analyzed for nitrate using colorimetry following Doane & Horwath (2003). Total nitrate leached was calculated by adding concentrations of all three extracts, back calculating to determine mass of NO_3^- per 50 g bag and converting to amount of nitrate leached per surface area of the puck, which was extrapolated to the field scale using equation 2 (Woodward et al., 2022):

$$(2) \quad \text{PLN} = (M_{\text{NO}_3-\text{N}}) \div A_p \times 10 ,$$

where PLN is potentially mineralizable NO_3^- (kg ha^{-1}), $M_{\text{NO}_3-\text{N}}$ is the mass of NO_3^- in each resin puck (g) and A_p is the area of the resin puck (0.00466 m^2). At resin installation and removal, soil samples were taken at 0-15, 15-30 and 30-60 cm, to attain residual soil SIN.

6.4 Soil organic carbon and nitrogen pools

Soil organic C and N pools (DOC, DON, MBC, and MBN) were measured using the chloroform fumigation extraction method described in Horwath and Paul (1996). Briefly, 6 g of field moist soil (0-15 and 15-30 cm) sieved to 4 mm was fumigated in a desiccator for 24 hours using 30 mL of chloroform (CHCl_3) in a beaker with boiling chips. After 24 hours, samples were shaken in 30 ml of 0.5 M K_2SO_4 for 1 hour and then filtered with Q5 filter paper. Another 6 g of soil was extracted similarly as the unfumigated control. Extracts were stored at -20°C until analysis for DOC and DON on a TOC/TN analyzer (Vario TOC cube, Elementar). Microbial biomass carbon and nitrogen were calculated as the difference in dissolved C and N fractions, respectively, between the fumigated and unfumigated samples, divided by a conversion factor of 0.35 and 0.68, respectively (Horwath & Paul, 1996).

6.5 Soil microbial community and function

Soil microbial community structure was characterized by using phospholipid fatty acid (PLFA) analysis (by Ward Laboratories – Kearney, NE) using a chloroform-methanol lipid extraction and gas

chromatography (Buyer & Sasser, 2012). Soil microbial activity was estimated through the potential activity of C cycling enzymes (β -Glucosaminidase and β -Glucosidase) and was analyzed (by Ward Laboratories – Kearney, NE) using substrate addition and incubation (Moscatelli et al., 2012; Parham & Deng, 2000).

6.6 Soil organic matter fractions

Soil organic matter fractions (MAOM and POM) were measured on 10 g of 4 mm, air-dried soil via aggregate dispersion and particle size fractionation on a wet sieve (Brewer et al., 2022). First, soil was shaken with 30 ml of 5% sodium hexametaphosphate and 5 glass beads for approximately 20 hours on a reciprocal shaker to disperse aggregates. After this, the soil slurry was poured on top a 53 μ m sieve on an automatic soil sieve with rainfall simulator (Fritsch Analysette 3 Pro – Idar-Obstein, Germany) and shaken with the rainfall simulator until the water ran clear (\sim 30 s). The <53 μ m (MAOM) and >53 μ m (POM) fractions were oven dried at 60 °C, weighed, ground, and analyzed for total organic carbon (TOC) and total organic nitrogen (TON) via combustion (ECS 4010, Costech Analytical Technologies, Inc). Concentration of carbon in each fraction (MAOC or POC) (X_i) was calculated using equation 3:

$$(3) \quad X_i \text{ (mg C g}^{-1}\text{OD soil)} = \frac{F_i \text{ (g)} \times \% C_i / 100}{\text{Soil}_{OD} \text{ (g)}} \times 1000$$

where F_i is the OD weight of each i-level fraction (either POM or MAOM) in grams, % C_i is the percent carbon of each i-level fraction and soil_{OD} is the total OD weight of soil recovered after the running the protocol. The OD weight recovered was between 98 and 102% of the initial air-dried soil weight. Concentration of nitrogen in each fraction (MAON and PON) was calculated in the same manner.

7 Statistical analysis

All statistical analysis was completed in R version 4.3.0. We apportioned the variance of response variables to fixed effects (winter treatment) and random effects (plot, block) and the function ‘lme’ in the R package ‘nlme’ was used to construct linear mixed-effect models. For soil chemical and biological

composition data, models were run on each depth at each time point separately. For all other response variables with multiple sampling timepoints in a year, date was included as a fixed effect with its respective interaction terms and different depths of soil samples were run in separate models due to highly significant depth fixed effects. For response variables only measured at single timepoint in a year (PMN, PLN, TC, TN, MAOC, POC) the model included treatment, depth, and their interaction.

Potential outliers were detected using a graphical boxplot display. Then, the `rosnerTest` was run with the suspected number of outliers, k , and they were removed if they were confirmed, and it made biological sense. Response variables were transformed if residual plots indicated violated assumptions of heterogeneity and normality of residuals. Actinomycetes biomass (Year 2 NutrientU) and PMN (Year 2 CropT) and were transformed using a \sqrt{x} . Potentially leachable nitrate (Year 2), MAOC (Year 4), gram (+) / gram (-) ratio (Year 2 CropT and NutrientU) and saprophytic biomass, total fungal biomass and fungal/bacterial ratio (Year 3 NutrientU) were transformed using $\log(x)$. Arbuscular biomass was transformed with $1/\sqrt{x}$ at CropT and with $1/x$ at NutrientU in Year 3. Response variables were analyzed with analysis of variance (ANOVA) at a 5% type-1 error probability level. Adjusted post-hoc Tukey tests were used for mean comparisons using the R package ‘emmeans’.

A heatmap was created by using `melt()` in `reshape2` package and `scale()`, to reshape and standardize the PLFA data matrix for use in `ggplot`, respectively.

8 Results

Soil physical characteristics did not differ among treatments. Bulk density was not impacted by cover crops or grazing in either Year 2 or 3 of ICLS implementation (Figure 3A and B). The MWD of aggregates and large macroaggregates fraction were not impacted by treatment ($p = 0.6510$, $p = 0.2571$, respectively) or depth ($p = 0.1431$, $p = 0.8721$, respectively) in Year 3 (Figure 3C and D). The small macroaggregate fraction decreased with depth, while the microaggregate fraction increased with depth, though both were not impacted by treatment (Figure 3E and F).

Some soil chemical properties were altered with grazing (notably N and pH), while most were not. Soil pH was lowered by cover crop, whether grazed or not, in Year 2 at crop planting at 0-15 cm, in the whole topsoil profile at peak nutrient uptake, and in Year 3 at crop planting and peak nutrient uptake (Table 1). Gravimetric water content was higher with cover crop, whether grazed or not, early in the season in Year 2, but this effect was not measured at other timepoints. Salinity (EC) was low among all treatments but slightly higher in soils from the ungrazed and grazed CC plots at 0-15 cm in Year 2, but not Year 3. Soil CEC, P, K, Na, Ca and Mg were not significantly altered by treatment at any timepoint in either year.



Figure 3. Soil physical properties.

Panel A and B - Bulk density in Year 2 and 3 of ICLS implementation, respectively. **Panel C, D, E and F** - Mean weight diameter of aggregates and percent of large macroaggregates, small macroaggregates and microaggregates in Year 3, respectively. NS is not significant at a p-value of 0.05; (*) is significant at a p-value < 0.05; (**) is significant at a p-value < 0.01; and (***) is significant at a p-value < 0.001.

Yr	Time	Depth	Trt	pH	GWC	EC (mmho cm ⁻¹)	CEC (Meq 100g ⁻¹)	P (ppm)	K (ppm)	Na (ppm)	Ca (ppm)	Mg (ppm)
2	Crop Plant	0-15 cm	fallow	7.58 (0.03) b	0.09 (0.01) a	0.30 (0.03) a	19.6 (0.7)	56.7 (3.7)	327 (29)	84 (4.8)	1629 (64)	1231 (35)
			UCC	7.23 (0.04) a	0.11 (0.01) b	0.44 (0.07) b	19.1 (0.5)	55.2 (2.2)	361 (37)	83 (7.6)	1574 (47)	1188 (23)
			GCC	7.19 (0.03) a	0.11 (0.00) ab	0.47 (0.05) b	19.9 (0.8)	59.9 (2.1)	374 (24)	95 (4.1)	1650 (71)	1233 (45)
		15-30 cm	fallow	7.54 (0.09)	0.12 (0.00)	0.16 (0.03)	19.0 (0.6)	33.6 (0.1)	193 (10)	93 (4.8)	1559 (51)	1231 (33)
			UCC	7.51 (0.04)	0.12 (0.00)	0.14 (0.02)	18.9 (0.3)	31.2 (1.8)	183 (16)	85 (7.0)	1559 (30)	1238 (15)
			GCC	7.56 (0.02)	0.12 (0.00)	0.12 (0.02)	19.8 (0.7)	36.7 (4.2)	198 (20)	89 (3.6)	1633 (62)	1291 (40)
	Peak Nutr	0-15 cm	fallow	7.50 (0.07) b	0.08 (0.02)	0.39 (0.02) a	20.8 (0.6)	55.3 (3.9)	340(20)	100 (4.8)	1745 (47)	1294 (38)
			UCC	7.13 (0.07) a	0.08 (0.01)	0.56 (0.05) b	20.6 (0.5)	52.5 (2.8)	363 (54)	97 (11.5)	1699 (39)	1283 (24)
			GCC	7.16 (0.02) a	0.06 (0.00)	0.54 (0.05) ab	21.0 (0.3)	56.9 (2.4)	382 (14)	95 (3.6)	1711 (31)	1320 (19)
		15-30 cm	fallow	7.48 (0.06) b	0.14 (0.01)	0.28 (0.03)	20.0 (0.7)	30.9 (2.9)	212 (25)	117 (12.2)	1648 (69)	1290 (33)
			UCC	7.29 (0.07) a	0.14 (0.01)	0.25 (0.03)	19.4 (0.4)	30.0 (2.0)	185 (27)	97 (8.4)	1577 (33)	1274 (23)
			GCC	7.26 (0.02) a	0.11 (0.00)	0.25 (0.02)	21.0 (0.6)	30.6 (3.0)	217 (16)	93 (3.8)	1737 (55)	1367 (38)
3	Crop Plant	0-15 cm	fallow	7.51 (0.04) b	0.09 (0.01)	0.64 (0.08)	22.1 (0.4)	63.4 (2.9)	388 (14)	135 (4.0)	1837 (57)	1364 (18)
			UCC	7.38 (0.03) a	0.08 (0.01)	0.66 (0.05)	21.9 (0.9)	70.3 (6.7)	406 (30)	149 (9.4)	1820 (86)	1332 (60)
			GCC	7.36 (0.02) a	0.08 (0.01)	0.55 (0.04)	21.4 (0.5)	63.4 (4.4)	398 (27)	124 (8.6)	1751 (44)	1336 (30)
		15-30 cm	fallow	7.48 (0.03) b	0.09 (0.01)	0.45 (0.02)	22.7 (0.5)	43.8 (3.7)	305 (23)	123 (3.6)	1866 (46)	1448 (31)
			UCC	7.38 (0.03) a	0.08 (0.00)	0.50 (0.09)	23.2 (0.3)	51.6 (6.2)	351 (17)	125 (14.3)	1906 (29)	1466 (22)
			GCC	7.45 (0.02) ab	0.08 (0.01)	0.43 (0.04)	23.4 (0.9)	52.1 (3.2)	367 (24)	124 (9.1)	1926 (85)	1476 (52)
	Peak Nutr	0-15 cm	fallow	7.54 (0.05) b	0.08 (0.00)	0.54 (0.05)	22.5 (0.6)	69.1 (2.5)	415 (16)	145 (2.9)	1903 (56)	1362 (38)
			UCC	7.23 (0.05) a	0.08 (0.00)	0.86 (0.21)	22.8 (0.7)	70.8 (5.1)	444 (21)	153 (24.1)	1888 (30)	1392 (10)
			GCC	7.10 (0.04) a	0.07 (0.01)	0.82 (0.16)	21.5 (0.3)	77.9 (3.2)	442 (33)	147 (2.0)	1757 (60)	1309 (43)
		15-30 cm	fallow	7.45 (0.05) b	0.09 (0.00)	0.40 (0.03)	22.0 (0.6)	40.8 (3.2)	246 (21)	125 (6.5)	1829 (54)	1404 (33)
			UCC	7.24 (0.02) a	0.09 (0.00)	0.50 (0.09)	23.1 (1.0)	40.3 (2.6)	245 (12)	122 (15.0)	1914 (91)	1483 (56)
			GCC	7.19 (0.07) a	0.09 (0.01)	0.57 (0.08)	22.3 (0.5)	42.7 (1.5)	265 (25)	127 (7.4)	1855 (34)	1421 (30)

Table 1. Soil chemical properties.

Soil chemical property means and standard errors (in parentheses) from Year 2 (2020) and Year 3 (2021) of ICLS implementation at crop planting (Crop Plant) and peak crop nutrient uptake (Peak Nutr). Lowercase letters represent significant differences between treatments within year, sampling timepoint and depth. UCC = ungrazed CC; GCC = grazed CC.

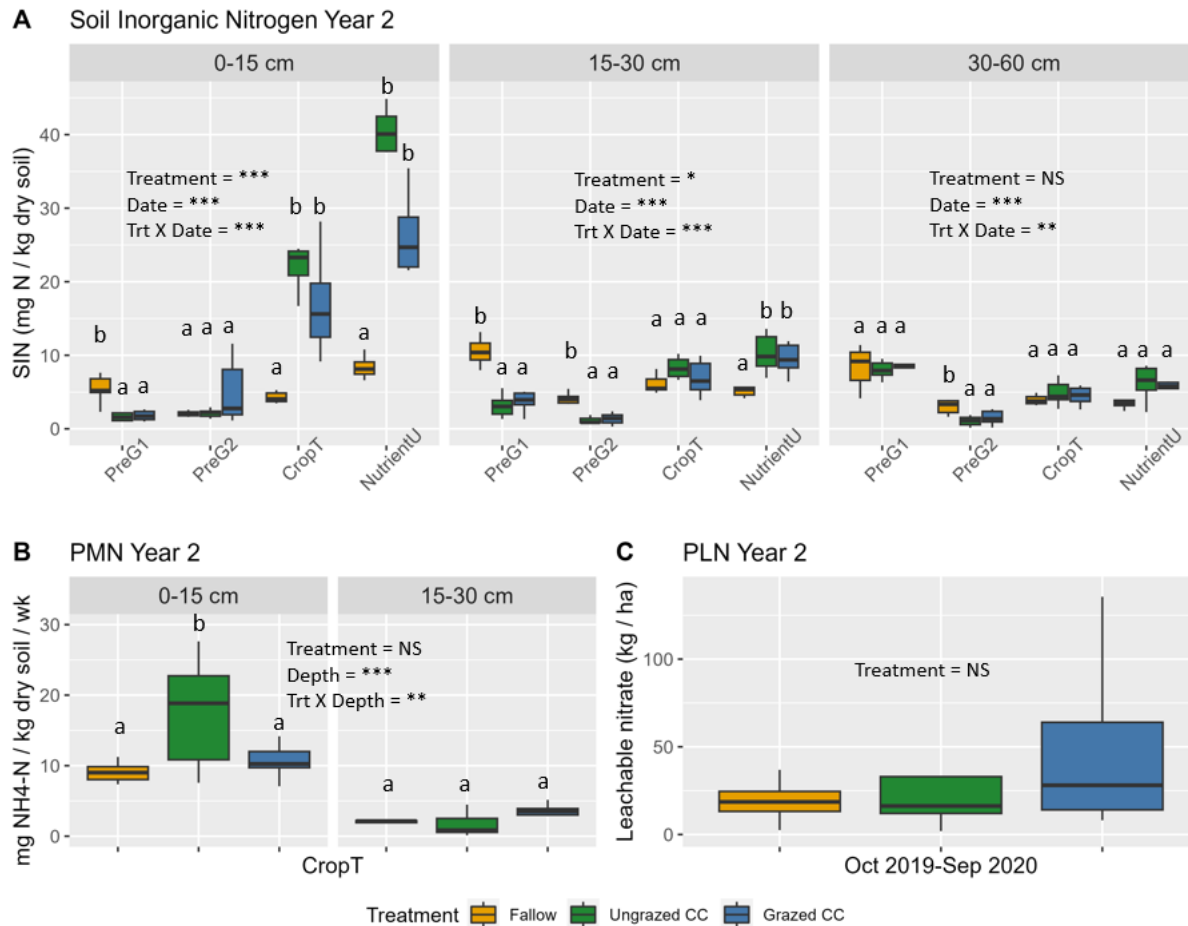


Figure 4. Soil inorganic nitrogen pools and processes in 2020.

Panel A – Soil inorganic nitrogen (SIN) in Year 2 of ICLS. Year 2 had four timepoints which are represented on the x-axis: pregraze 1 (PreG1), pregraze 2 (PreG2), crop transplanting (CropT) and peak crop nutrient uptake (NutrientU). **Panel B and C** - Potentially mineralizable nitrogen (PMN) and potentially leachable nitrate (PLN) in Year 2, respectively. Lowercase letters represent significant differences between treatments within year, sampling timepoint and depth. NS is not significant at a p-value of 0.05; (*) is significant at a p-value < 0.05; (**) is significant at a p-value < 0.01; and (***) is significant at a p-value < 0.001.

Grazing and cover crops significantly altered soil inorganic N pools (SIN). In Year 2 of ICLS implementation, the SIN pool generally increased over time at all depths (Figure 4A). Prior to grazing in Year 2, grazed and ungrazed CC had less SIN at 0-15 and 15-30 cm than fallow, with no treatment differences observed at 30-60 cm. After the first grazing event, this cover crop effect persisted with grazed and ungrazed CC having significantly less SIN at 15-30 and 30-60 cm. No treatment differences were observed at 0-15 cm. At crop transplanting, the cover crop effect reversed with grazed CC and ungrazed

CC plots having greater SIN than fallow at 0-15 cm (~ 23 in CC treatments compared to 4 mg N kg⁻¹ dry soil in fallow treatment). Soil collected at this time point shows that ungrazed CC soil had greater PMN at 0-15 cm than the other two treatments (Figure 4B). At peak nutrient uptake, the same cover crop effect persisted with grazed CC and ungrazed CC plots having greater SIN than fallow at both 0-15 cm and 15-30 cm, with no statistical differences at 30-60 cm. Despite these cover crop effects, there were no significant differences in potentially leachable N between all the treatments (Figure 4C).

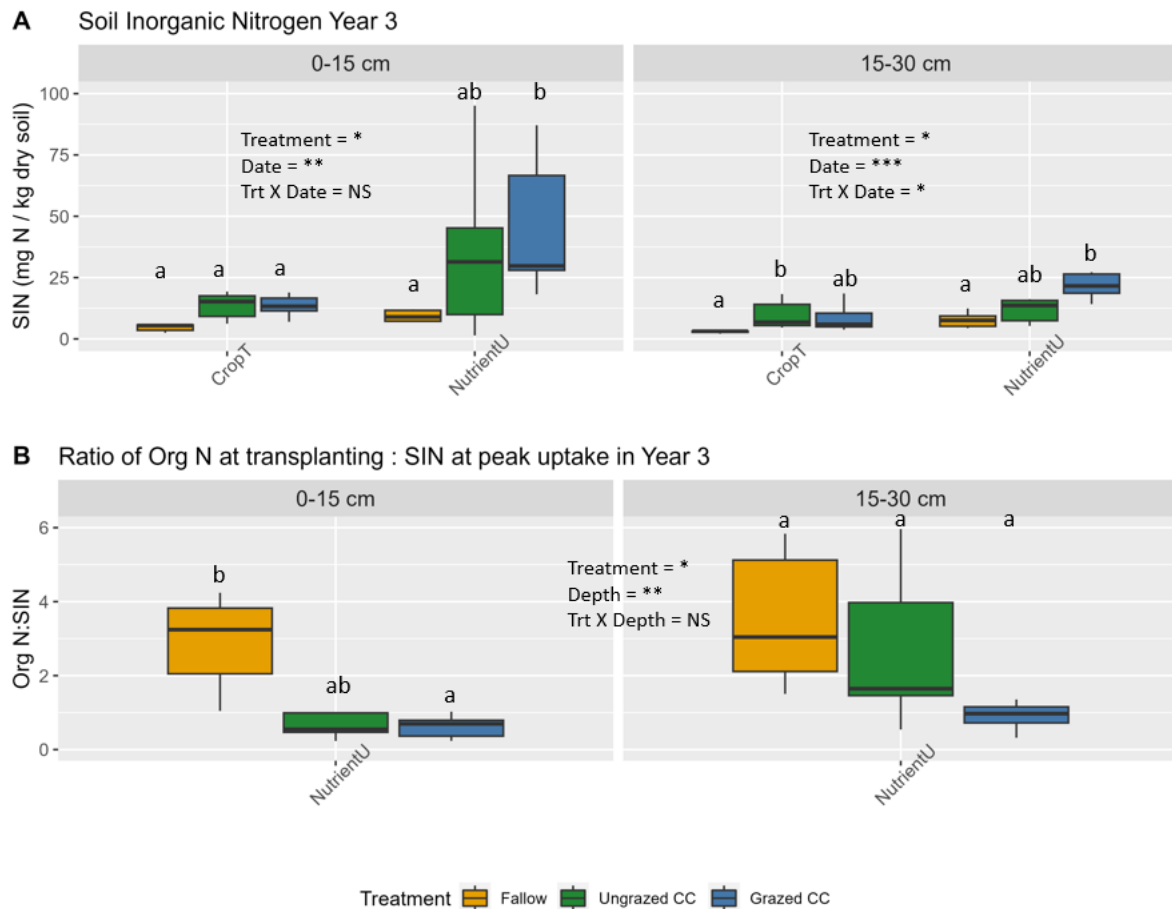


Figure 5. Soil inorganic nitrogen pools and processes in 2021.

Panel A – Soil inorganic nitrogen (SIN) in Year 3 of ICLS implementation. Year 3 had two timepoints which are represented on the x-axis: crop transplanting (CropT) and peak nutrient uptake (NutrientU). **Panel B** - The ratio of organic N (DON + MBN) at crop transplanting to SIN at peak nutrient uptake in Year 3, with a ratio close to 1 representing a system with N in the right form at the right time. Lowercase letters represent significant differences between treatments within year, sampling timepoint and depth. NS is not significant at a p-value of 0.05; (*) is significant at a p-value < 0.05; (**) is significant at a p-value < 0.01; and (***) is significant at a p-value < 0.001.

In Year 3, SIN in the topsoil generally increased over time (Figure 5A), similar to Year 2. At crop transplanting, there were no treatment differences at 0-15 cm, but at 15-30 cm soils under ungrazed CC had about three times greater SIN than fallow (9.24 compared to 3.20 mg N kg⁻¹ dry soil; $p = 0.0394$). At peak nutrient uptake, at 0-15 cm, grazed CC soil had about four times more SIN available compared to fallow (45.57 compared to 10.93 mg N kg⁻¹ dry soil; $p = 0.0442$), whereas the ungrazed CC plots had average SIN concentrations of 35.26 mg N kg⁻¹ dry soil. At the 15-30 cm depth, SIN in grazed CC plots was about three times greater than fallow (26.57 compared to 7.92 mg N kg⁻¹ dry soil; $p = 0.0394$). When looking at the fraction of organic N at transplanting to inorganic N at peak uptake, a proxy for PMN, grazed plots had a lower ratio than fallow at 0-15 cm and closest to 1 (0.62 ± 0.31 and 0.91 ± 0.38 at 0-15 and 15-30 cm, respectively) (Figure 5B). In summary, grazing increased SIN compared to fallow, without increases in N losses through leaching.

Dissolved organic carbon in the topsoil (0-15 and 15-30 cm) was not altered by grazing of CC compared to ungrazed CC or fallow at any timepoint either 2 (2020) or 3 (2021) years after treatment establishment (Figure 6A and B). In Year 2, DOC in the topsoil increased, while DON decreased over time similarly for all treatments (Figure 6A and C). In Year 2, at pregraze 1, and before any grazing event that year, grazed CC had significantly lower DON at 0-15 cm than fallow ($p = 0.0299$), showing a potential residual grazing effect from Year 1. At crop transplanting, the trend reversed and soils under grazed CC had significantly higher DON than fallow soils at 15-30 cm (13.52 compared to 8.30 $\mu\text{g N g}^{-1}$ OD soil; $p = 0.0168$) with ungrazed CC being indistinguishable from both. At peak crop nutrient uptake, the cover crop effect persisted but instead the ungrazed CC treatment led to greater DON at 0-15 cm compared to fallow ($p = 0.0436$) with grazed CC being indistinguishable from both (Figure 6C).

In Year 3 after treatment establishment, DOC at 0-15 cm did not vary over time, while DON increased from crop transplanting to peak nutrient uptake (Figure 6B and D). At lower depth (15-30 cm), DOC decreased from crop transplanting to peak nutrient uptake, while DON did not vary over time. In Year 3, at peak nutrient uptake, grazed CC plots had about double the concentration of DON than the fallow

plots at 0-15 and 15-30 cm (48.6 compared to 25.8 $\mu\text{g N g}^{-1}$ OD soil; $p = 0.0412$ and 23.8 compared to 11.3 $\mu\text{g N g}^{-1}$ OD soil; $p = 0.0071$, respectively), with ungrazed CC being indistinguishable from both (Figure 6D). In summary, three years of cover crop grazing did not alter soil DOC levels from ungrazed CC while DON tended to be significantly higher in soils under grazed CC compared to fallow.

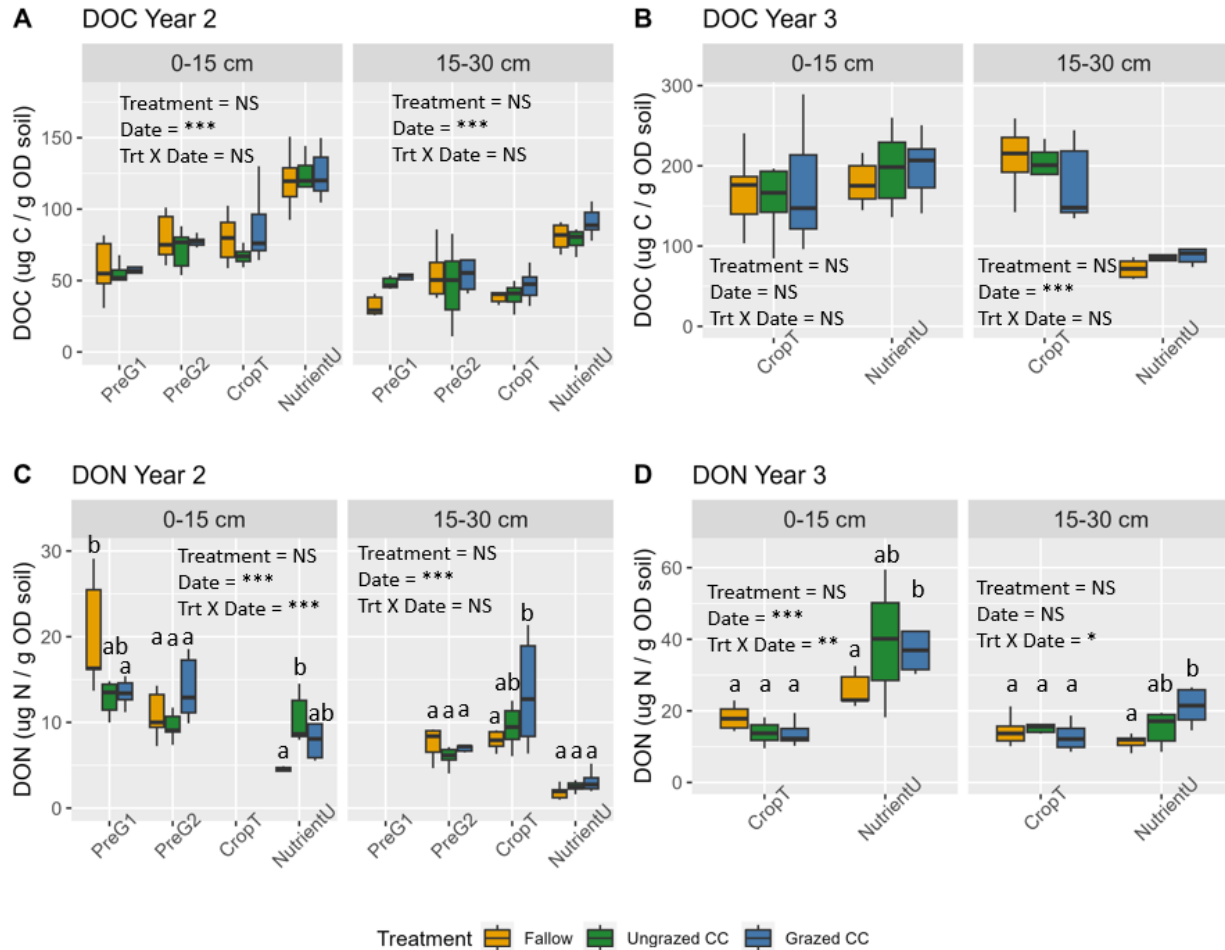


Figure 6. Soil dissolved organic carbon (DOC) and dissolved organic nitrogen (DON).

Panel A and B – DOC in Year 2 and 3 of ICLS implementation. **Panel C and D** – DON in Year 2 and 3 of ICLS implementation. Year 2 has four timepoints which are represented on the x-axis: pregraze 1 (PreG1), pregraze 2 (PreG2), crop transplanting (CropT) and peak nutrient uptake (NutrientU), while Year 3 has only the last two timepoints. Lowercase letters represent significant differences between treatments within year, sampling timepoint and depth. NS is not significant at a p-value of 0.05; (*) is significant at a p-value < 0.05; (**) is significant at a p-value < 0.01; and (***) is significant at a p-value < 0.001.

In Year 2 of ICLS implementation, MBC in the topsoil changed over time in a sinusoidal trend while MBN was generally lower later in the season (Figure 7A and C). There was no difference in MBN

concentration between winter treatments at any timepoint in Year 2 (Figure 7A and C). There was notably less MBC in grazed CC plots than ungrazed CC plots before any grazing in Year 2 at 15-30 cm (73.9 compared to 153.3 $\mu\text{g C g}^{-1}$ OD soil; $p = 0.0133$), showing a potential residual grazing effect from Year 1. At crop transplanting, this changed and grazed CC had significantly greater MBC than fallow at 0-15 cm (464 compared to 351 $\mu\text{g C g}^{-1}$ OD soil; $p = 0.0426$), with ungrazed CC not being distinguishable from the two. By peak nutrient uptake, this trend was no longer apparent (Figure 7A).

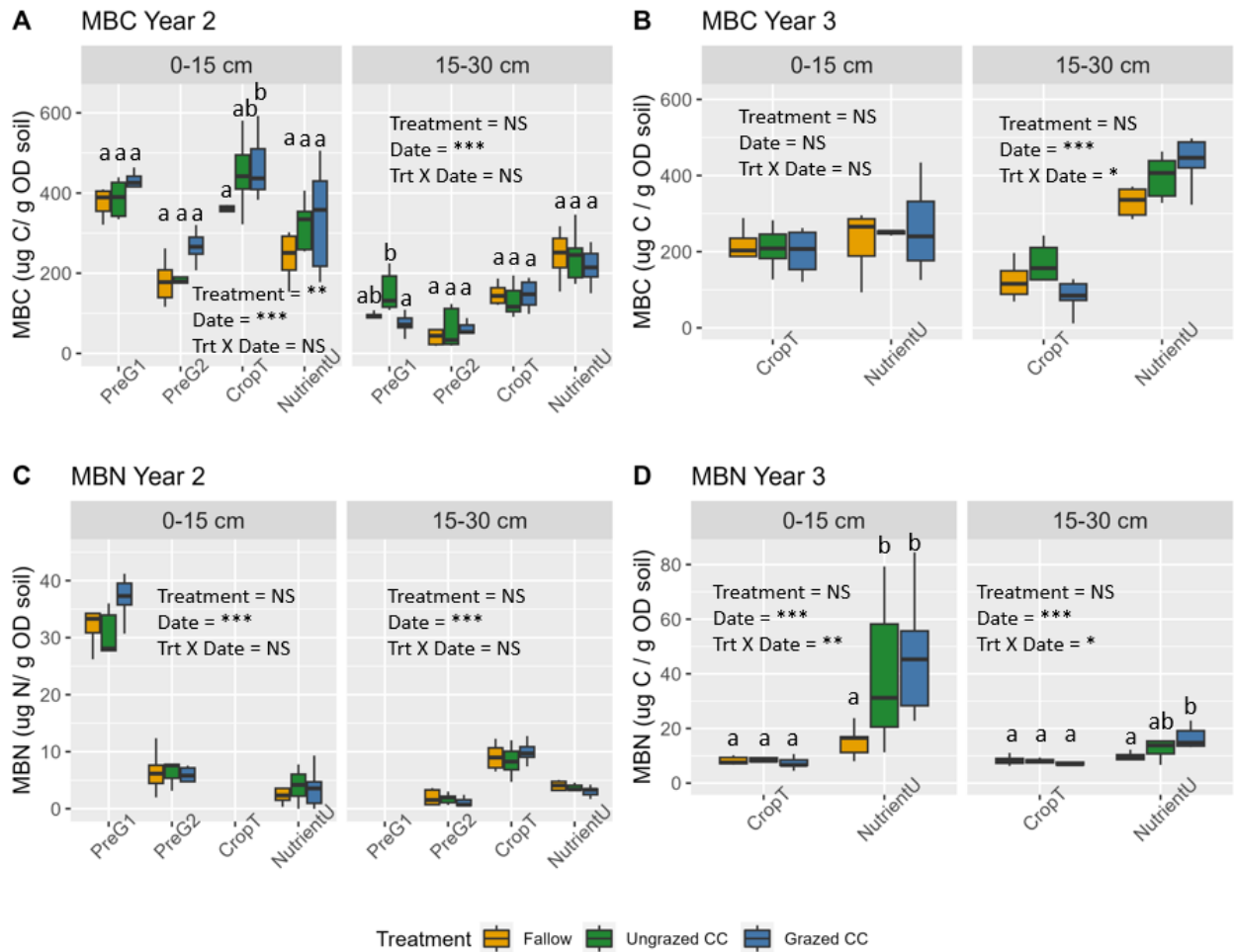


Figure 7. Soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN). **Panel A and B** – MBC in Year 2 and 3 of ICLS implementation, respectively. **Panel C and D** – MBN in Year 2 and 3 of ICLS implementation, respectively. Year 2 has four timepoints which are represented on the x-axis: pregraze 1 (PreG1), pregraze 2 (PreG2), crop transplanting (CropT) and peak nutrient uptake (NutrientU), while Year 3 has only the last two timepoints. Lowercase letters represent significant differences between treatments within year, sampling timepoint and depth. NS is not significant at a p-value of 0.05; (*) is significant at a p-value < 0.05; (**) is significant at a p-value < 0.01; and (***) is significant at a p-value < 0.001.

In Year 3, MBC and MBN increased over time from crop transplanting to peak nutrient uptake, with significant differences across treatments found only in the MBN pool (Figure 7B and D). At crop transplanting, MBC was lower at 15-30 cm with grazing compared to no grazing (78.7 grazed CC; 169.8 ungrazed CC $\mu\text{g C g}^{-1}$ OD soil; $p = 0.46276$) with this trend reversing at peak crop nutrient uptake (Figure 7B). At peak nutrient uptake, cover crops doubled the concentration of MBN at 0-15 cm in both the grazed and ungrazed CC treatments compared to fallow and CC grazing doubled the concentration of MBN compared to fallow at 15-30 cm (16.61 compared to 8.99 $\mu\text{g C g}^{-1}$ OD soil; $p = 0.0175$) (Figure 7D). Taking the two years together, grazing did not contribute to a clear trend in concentrations of MBC, but it increased MBN later in the crop stage compared to fallow.

The MBC/MBN ratio was not impacted by treatment (Figure 8C and D) while the DOC/DON ratio tended to be lower with grazed and ungrazed cover crops at peak nutrient uptake than fallow (Figure 8A and B). The DOC/DON ratio increased at both 0-15 and 15-30 cm as the season progressed in both years. The MBC/MBN ratio increased over time in Year 2 at both depths but decreased over time in Year 3 at 0-15 cm.

Although shifts in individual microbial groups due to grazing and cover crops were generally not significant, there were differences in ratios of functional groups. Grazing tended to decrease the fungal/bacterial ratio with the ratio being 0.26, 0.13, and 0.09 in fallow, ungrazed CC and grazed CC plots, respectively, in Year 2 at crop transplanting ($p = 0.004704$) (Figure 9A). At the final timepoint (Year 3, peak nutrient uptake), the fungal/bacterial ratio was 0.23, 0.11 and 0.03 in fallow, ungrazed CC and grazed CC plots respectively ($p = 0.04755$). Grazing tended to increase the gram (+) / gram (-) ratio. At the final timepoint, the gram (+) / gram (-) ratio was 1.61, 1.72 and 2.30 in fallow, ungrazed CC and grazed CC plots respectively ($p = 0.06829$). There were no clear trends in potential activity of extracellular enzymes.

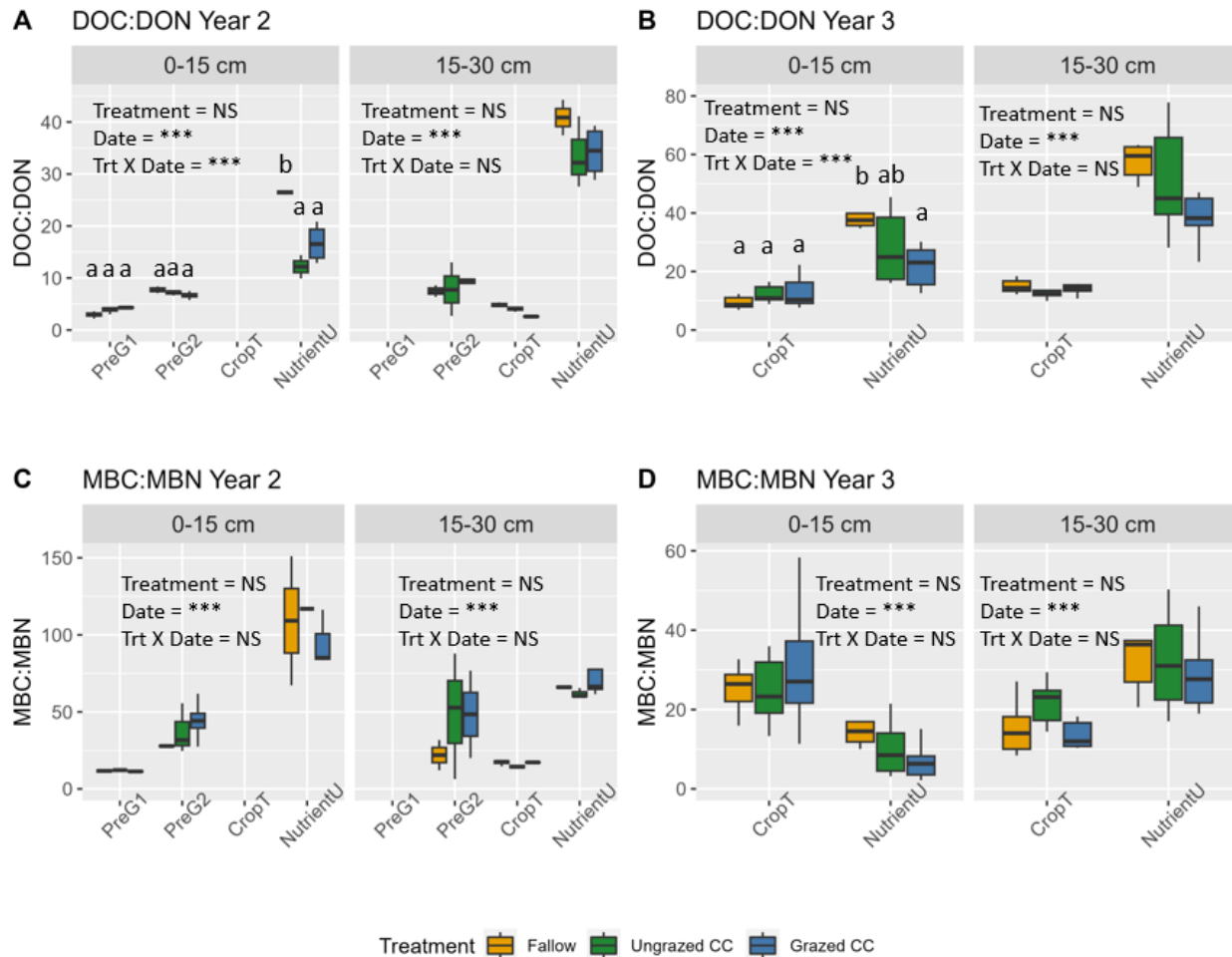


Figure 8. Soil organic carbon and nitrogen ratios.

Panel A and B – Dissolved organic carbon / nitrogen ratio (DOC/DON) in Year 2 and 3, respectively. **Panel C and D** - Microbial biomass carbon / nitrogen ratio (MBC/MBN) in Year 2 and 3, respectively. Year 2 has four timepoints which are represented on the x-axis: pregraze 1 (PreG1), pregraze 2 (PreG2), crop transplanting (CropT) and peak nutrient uptake (NutrientU), while Year 3 has only the last two timepoints. Lowercase letters represent significant differences between treatments within year, sampling timepoint and depth. NS is not significant at a p-value of 0.05; (*) is significant at a p-value < 0.05; (**) is significant at a p-value < 0.01; and (***) is significant at a p-value < 0.001.

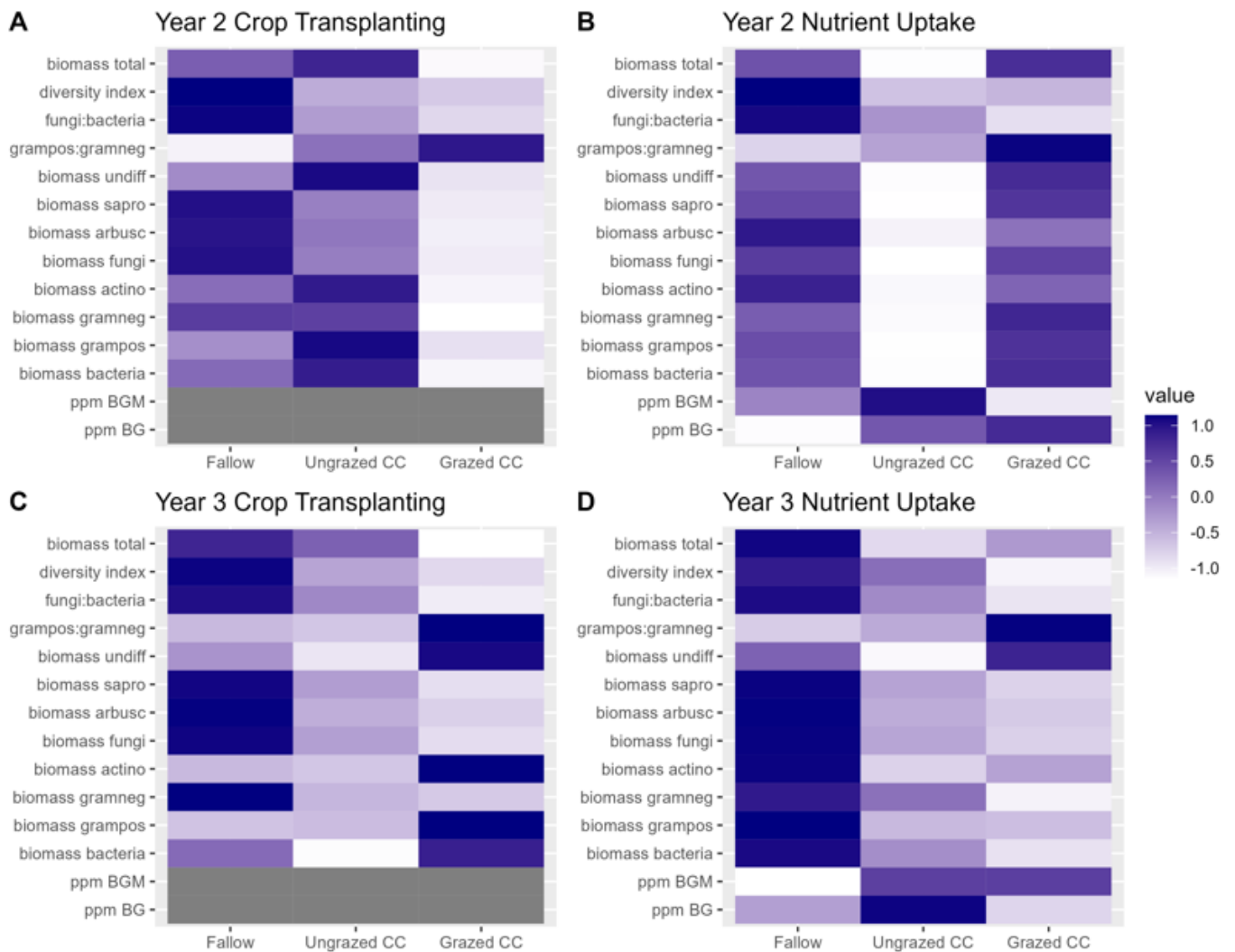


Figure 9. Soil microbial community composition and functions heatmaps using z scores of means. **Panel A and B** - Microbial community composition in Year 2 at crop transplanting and peak crop nutrient uptake, respectively. **Panel C and D** - Microbial community composition in Year 3 at crop transplanting and peak crop nutrient uptake, respectively. Grey represents data that was not collected (β -glucosidase (ppm BG) and β -glucosaminidase (ppm BGM)) at crop transplanting.

At the end of our experiment, there was no treatment effect on total soil C, total soil N, or MAOC at any depth (Figure 10A, B, C, respectively). Carbon in the POM fraction was lower at 0-15 cm in the plots with cover crops (Figure 10D). Soil C, N, MAOC and POC all decreased with depth.

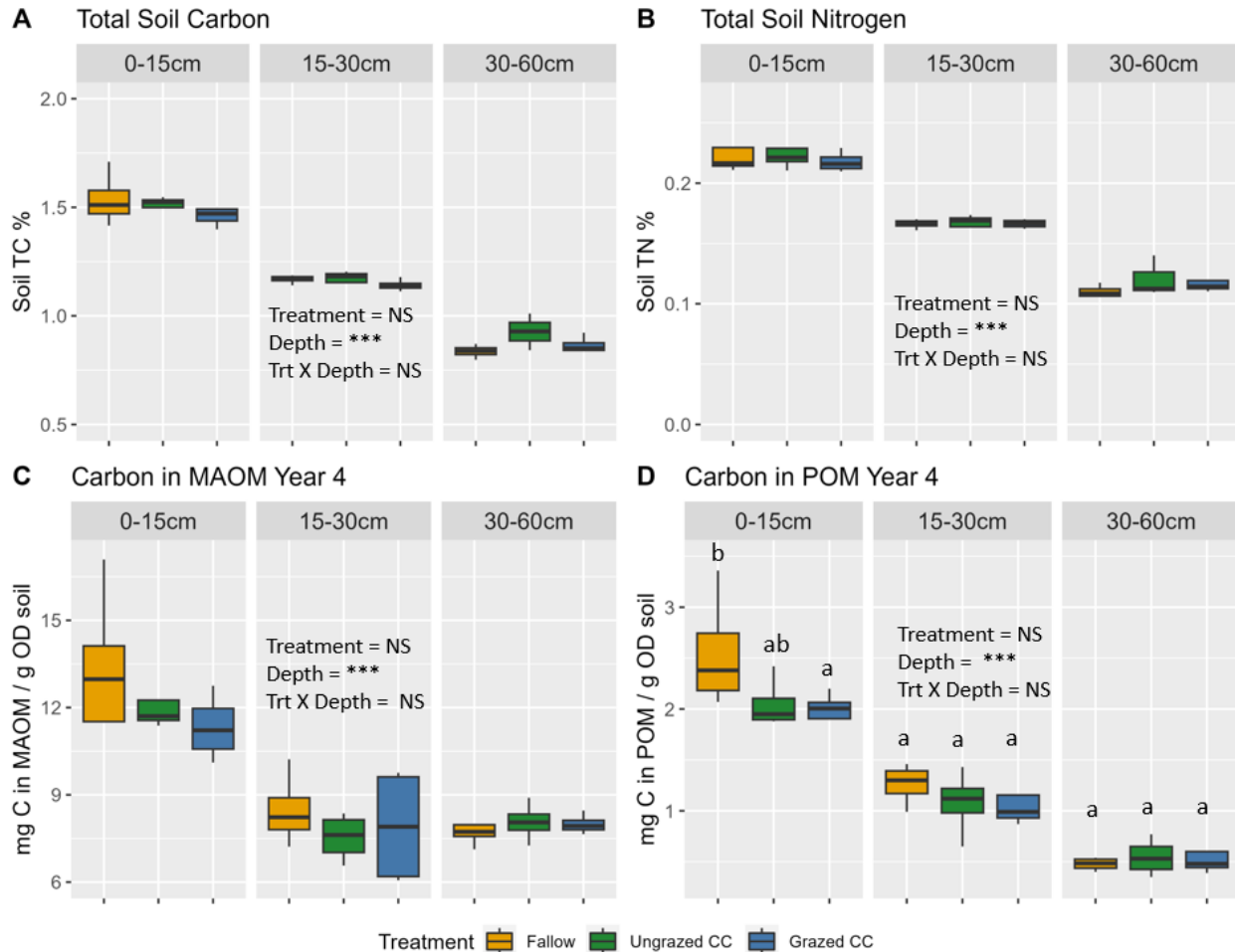


Figure 10. Soil organic matter fractionation at end of experiment.

Panel A and B - Total soil carbon (C) and soil nitrogen (N) in Year 4, respectively. **Panel C and D** – Carbon in particulate and mineral-associated organic matter fraction in Year 4, respectively. Lowercase letters represent significant differences between treatments within year, sampling timepoint and depth. NS is not significant at a p-value of 0.05; (*) is significant at a p-value < 0.05; (**) is significant at a p-value < 0.01; and (***) is significant at a p-value < 0.001.

9 Discussion

9.1 Field trial implementation outcomes

We observed variability in grazing implementation and cover crop responses over the four years of the experiment. Grazing intensity varied during every event based on weather constrictions around timing and availability of sheep. In Year 2, there was a high intensity graze (1 cm sward height post graze), followed by a moderate intensity graze (20 cm sward height post graze). In Year 3, there was a moderate

intensity graze (10 cm sward height post graze) followed by a high intensity graze (6 cm sward height post graze). Cover crops in our trial did not accumulate enough biomass to graze until about mid-February. Warmer and longer days post grazing allowed for CC regrowth and a second grazing event about 5-6 weeks later in Years 2 and 3. We observed that the sheep selectively grazed certain species, but if left long enough would consume most vegetation in the plots. They preferentially grazed weeds such as shepherd's purse and mallow while avoiding fava beans and daikon radish, likely because these sheep didn't have experience grazing CC before our trial but had learned to eat common weeds found in California. We also observed less weed pressure in our grazed plots during the crop and changes in weed community composition away from the dominant monocot weed (*Echinochloa crus-galli*) towards dicots (data not shown). Grazing may have stimulated earlier germination of weeds, increased seed predation or otherwise impacted seed viability (Blubaugh et al., 2016; Mohler et al., 2018; Szwed et al., 2020). Multiple grazing events can also shift plant community composition (Smith et al., 2014) which we observed as the proportion of grasses in our CC stand increased post grazing while the legumes decreased. Grasses have a lower C/N ratio than legumes and may mineralize slower than mixes with legumes, which could lead to tie up of N and lower crop yields or, in our case, may have contributed to the mitigation of nitrate leaching potential (Finney et al., 2016; Hunter et al., 2019; Li et al., 2021).

9.2 Physicochemical outcomes

Integrated crop-livestock studies could take longer than 3 years to detect changes in soil health, particularly in physicochemical properties. Thus, we did not expect to see any shifts in soil physical properties, which was confirmed by no differences in soil compaction or structure. Similar to us, one review of 67 paired comparisons of \pm grazing in grasslands found that bulk density either increased or did not change with grazing (Piñeiro et al., 2010). One ICLS study under conventional tillage found that aggregate stability was unaffected by grazing, but soil penetration resistance (which we did not measure) was greater with grazing (Franzluebbers & Stuedemann, 2008b). Another paper found a higher MWD with ICLS that had been implemented for 30 years, but not with ICLS that had only been implemented for 3 years (Bansal

et al., 2022), so our experiment length may not have been longer enough to see an appreciable shift in MWD. However, it is also important to note that in the previously referenced study the grazing effect at 30 years is not distinguishable from the cover crop effect as there was not a control ungrazed CC to compare with the ICLS system (Bansal et al., 2022), highlighting the importance of having an ungrazed comparison in ICLS studies.

A no-till ICLS study in an acidic soil (pH 4.4-5.2) and humid subtropical climate over 9 years found no change in soil pH down to 40 cm with grazing compared to no grazing (Martins et al., 2014), as we saw in our experiment. Generally, in our trial, the cover crop effect was stronger than the grazing effect and seemed to drive the decrease in pH in our two cover crop treatments presumably because the cover crop root activity (which release hydrogen ions as they take up cations) didn't differ substantially between these two treatments. Another potential explanation for comparable pH's in the two cover crop treatments is that flash grazing doesn't remove substantial base cations from the system; if this was the case, we may have measured increased soil acidification in grazed CC compared to ungrazed CC. Soil type, tillage, climate, and study length may all play a role in outcomes on pH as another study looking at tilled ICLS in semiarid North Dakota (pH 6.0-7.0) found more acidification with grazing than no grazing after 11 years (Liebig et al., 2016).

9.3 Microbial community outcomes

Soil biological properties have been shown to have detectable responses to grazing sooner than physicochemical changes (Sekaran, Kumar, et al., 2021) and we expected the microbial community and functioning in the grazed CC treatment to shift away from decomposition functions and towards nutrient cycling functions. We expected the fungal/ bacterial ratio to be lower in the grazed compared to the ungrazed cover crop treatment, driven by less saprophytic and AM fungi (Ilmarinen et al., 2008) which was confirmed by our results. In our case, multiple grazing events of our CC mix selected for grass species, while decreasing prevalence of legumes, and this lower plant species richness, in addition to shifts in residue lability due to grazing, could have contributed to a lower fungal/ bacterial ratio (Bansal et al., 2022; Lange

et al., 2019). However, other ICLS research has found that grazing increased saprophytic fungal biomass at the 10-15 cm depth (Alves et al., 2023). In our study, the gram (+) / gram (-) ratio was higher in the grazed CC compared to the ungrazed treatment, suggesting the grazed system had greater disturbance and thus mineralization (Klump et al., 2009). But, specific outcomes on C and N cycling enzymes in our study were unclear. In contrast, one study found that sheep grazing decreased the activity of the C cycling enzyme (β -Glucosidase) (Tian et al., 2010), while a study on an agroforestry ICLS system saw an increase in this enzyme linked with higher water content (Sarto et al., 2020).

9.4 Labile carbon and nitrogen outcomes

In ICLS experiments, it is important to compare ungrazed CC to grazed CC to differentiate the effects of cover crops from grazing, particularly in regard to soil C and N outcomes. We aimed to understand how grazing of cover crops impacts bioavailable soil C and N pools and whether it is an effective practice for timely N release from cover crop. We observed variability in grazing impacts on labile C and N pools over the four years of the experiment.

We expected cover crops to increase labile C and N pools and decrease nitrate leaching compared to fallow. We found that cover crops decreased SIN during the cover crop phase, but increased SIN, MBC, MBN and DON in the cropping season. Cover crops increased MBN after 3 years, while MBC pools were only increased by cover crops in Year 2. Although cover crops didn't lead to an increase in total soil C, higher bacterial abundance has been measured as a result of cover cropping (Schmidt et al., 2018), which tracks with the higher MBC observed in our cover crop treatment. Similarly, one study investigating cover cropping in intensive vegetable cropping in Salinas, CA found that cover crops increased POX-C which correlated positively with MBC and MBN after 8 years. Decreased SIN during the spring was to be expected as cover crop mixtures can take up between 29 – 77 kg N ha⁻¹ (Kaye et al., 2019). Most experiments monitor nitrate leaching for only a portion of the year (Pare et al., 1995; Woodward et al., 2022) but we aimed to capture a full year of leaching to detect if leaching during summer irrigation contributed significantly to total N leaching. Although there was reduced SIN during cover crop growth, we didn't observe a reduction

in nitrate leaching. With cover crop addition, soils with higher sand content can have a greater reduction in nitrate leaching compared to other textures (Nouri et al., 2022). The lower leaching potential of the silt loam texture at our site combined with well managed irrigation through drip likely minimized deep percolation, and thus, cover crops provided less mitigation. Higher SIN during the crop phase compared to fallow suggests that the CC mixture and environmental conditions may have provided a good opportunity for balancing common tradeoffs in N cycling and availability in organic systems (White et al., 2017).

We expected grazing to increase bioavailable C and N pools compared to the ungrazed CC. Grazing at light to moderate intensities is associated with greater root turnover and root mass (Mapfumo et al., 2002; Mcinently et al., 2010) and moderate grazing intensities keep plants at stages with less lignin, which could decompose faster than plant residues under other grazing intensities or no grazing at all (J. M. Assmann et al., 2015). Both urine and manure are potential sources of nitrogen with varying rate of mineralization, which may help enhance bioavailable N (Hoogendoorn et al., 2010; Sørensen & Jensen, 1995). However, we found that, the cover crop effect was stronger than the grazing effect on the DON pool and DOC was not altered by grazing. Our results are in contrast to a study looking at CC grazing by chickens, which found greater DOC and DON in grazed plots than ungrazed (Zheng et al., 2020). In our experiment, DOC may not have been an effective indicator of potential increases in root exudates and turnover due to grazing because we did not sample the rhizosphere nor sample immediately after grazing. In one study, the effects of clipping on soil rhizosphere carbon became not significant 7 days after clipping (Hamilton & Frank, 2001), so we could have missed the window of detection.

Despite no differences in DOC, we expected to observe higher MBC and MBN in the grazed plots. However, the only time in which the grazing effect was stronger than the cover crop effect was in Year 2 before grazing when there was lower MBC in grazed plots than ungrazed plots. Impacts at this timepoint are likely a lingering grazing effect from Year 1, showing the rapid response of soil ecosystems to a first year of grazing. Similarly, a study by Franzluebbbers and Stuedemann (2015), found that grazing had minimal impacts on MBC, and hypothesized that grazing and conventional tillage do not interact to increase

MBC, while no-till has more potential because of decreased disturbance. Our system was highly disturbed with tillage at cover crop planting, crop planting and for weed control during the crop which could have limited the benefits of grazing as tillage could have greater control on soil effects than flash grazing CC (Mubvumba & DeLaune, 2023). In contrast, one study found that grazing winter cover crops increased MBC from 1162 to 1259 mg kg⁻¹ at 0-3 cm at samples taken in winter after 3 years (Franzluebbers & Stuedemann, 2008a). It is possible that our 0-15 cm fraction was not precise enough and a smaller depth stratification would have helped elucidate more nuanced responses of microbial biomass (Franzluebbers & Stuedemann, 2008a). Another study found that after 4 years of including a grazed pasture in rotation, MBC at 0-15 cm was greater than continuous corn (Tracy & Zhang, 2008). It is possible our study, in which we measured MBC in year 2 and 3, was not enough time to detect changes due to interannual variability in rainfall and other environmental factors. One study comparing grazed and ungrazed plots found that grazing did not alter the availability of N or P, though SOC was increased (Liebig et al., 2016). A lack of N impacts in that study could have been observed because this ICLS system did not include a legume cover crop, like ours did, which can fix nitrogen, becoming an N source.

Although the impacts of grazing compared to cover crops were modest, starker differences were observed when comparing grazed to fallow plots, displaying the potential for grazing to enhance the benefits of cover crops. We expected bioavailable C and N pools to be increased in the grazed CC plots compared to the fallow. We hypothesized that grazing would increase N in SOM at transplanting relative to in inorganic N and support adequate N availability in-season while lowering N loss potential. Grazing balanced organic N at transplanting to SIN at peak uptake, while minimizing loss potential, compared to fallow (Figure 5B). Cover crops alone may not optimize the tradeoff between N supply and retention whereas grazing magnified the benefits of cover crops for N availability in season (Figure 4 and 5). After 3 years of grazing, early in the season there was more SIN in ungrazed CC than grazed, but later at peak nutrient uptake, there was more SIN in grazed CC, suggesting that grazing times the release of N better than CC alone. There was ample organic N at transplanting that was released into the SIN pool by crop

peak nutrient uptake, suggesting grazing has the potential to optimize the timing of nutrient release from cover crops. With grazing, there was also a lower DOC/DON ratio, suggesting labile C was being decomposed via the MCP, leading to a ratio closer to that of MBC/MBN (10-20) (Figure 8). Though not significant, trends towards a lower MBC/MBN ratio in grazed compared to ungrazed (Figure 8) corroborated the decreased fungal/ bacterial ratio with grazing (Figure 9). Shifts in soil MBC/MBN ratio could be due to microbial community composition change, with fungi having a C/N ratio of ~ 14-16 and bacteria having a lower ratio (~ 9-11) (McGill et al., 1981). Globally, MBC/MBN only varies slightly with a mean of about 10 (Gao et al., 2022). The near universal consistency of the MBC/MBN ratio means any important changes due to grazing will likely be in relation to MBC and MBN pool size, rather than the ratio of the two, but the ratio could still verify shifts in PLFA results, like in our trial.

Most SIN differences we observed were in 0-30 cm likely partially because sheep urine is typically retained in the 0-15 cm depth (Williams & Haynes, 1994) and tillage was down to 25 cm, which homogenized the soil. We didn't see an increase in leaching at depth due to grazing, which is in accordance with Williams & Haynes (1994) where NO_3^- at depth was the same in both in sheep grazed and ungrazed pastures. In our trial, the average mean leachable nitrate was $32.6 \text{ kg N ha}^{-1}$ which is comparable to mean leaching values between $21.8 - 58.2 \text{ kg ha}^{-1}$ that were observed at Russell Ranch from October 2018 to March 2019, in which there was 632 mm of rainfall (Woodward et al., 2022). Thus, based on our results it appears that the bulk of leaching does occur in the rainy season.

9.5 Organic matter stabilization

We expected POM to be decreased with grazing and MAOM to be increased. However, mineral-associated organic matter wasn't altered by grazing. It likely takes longer than 4 years to observe changes in the MAOM fraction, which could explain why we didn't see a shift. However, there was a trend towards less % C in the POM fraction in grazed CC than fallow at 0-15 cm after 4 years of grazing, which could be due to fewer POM inputs or greater POM turnover with grazing. We removed large residue, like maize stubble pieces, before running the analysis on SOM fractions, but there was more large residue visually

apparent in the fallow and ungrazed plots than the grazed CC plots, suggesting the labile N inputs could have helped break down large pieces of POM. Another study similarly found that POC at 0-3 cm was lower with grazing than without grazing (Franzluebbers & Stuedemann, 2008a). Moreover, there could be smaller or fewer roots in the grazed plots, or a change in root C/N, which could all contribute to faster decomposition and less contribution to the POM pool. Additionally, all of these factors could be regulated by grazing intensity. A greenhouse study looking at the impacts of two clipping events to 4 cm that were 3 weeks apart (considered high intensity) on *Festuca campestris* concluded grazing redirects C flows to shoots as they regrow, leading to slowed elongation and decreased production of new roots, but not greater root death (McInenly et al., 2010). It is unknown how roots of legumes and grass differ in recalcitrance, but this knowledge would help us understand how grazing impacts the rate of decomposition of roots. Recalcitrant roots that slough off led to only short-term SOM stabilization (due to decreased decomposition) whereas less recalcitrant roots led to longer term SOM stabilization through microbial processing with less C lost through respiration, and eventually interaction with metals and minerals to form MAOM (Poirier et al., 2018). Although the mechanisms by which roots contribute to stabilization (recalcitrance, occlusion, and interaction with metals and minerals) and destabilization (priming effects) of C are known (Bailey et al., 2019), the balance of how root quality impact these two processes (Poirier et al., 2018), and how grazing impacts root quality, is all still unclear.

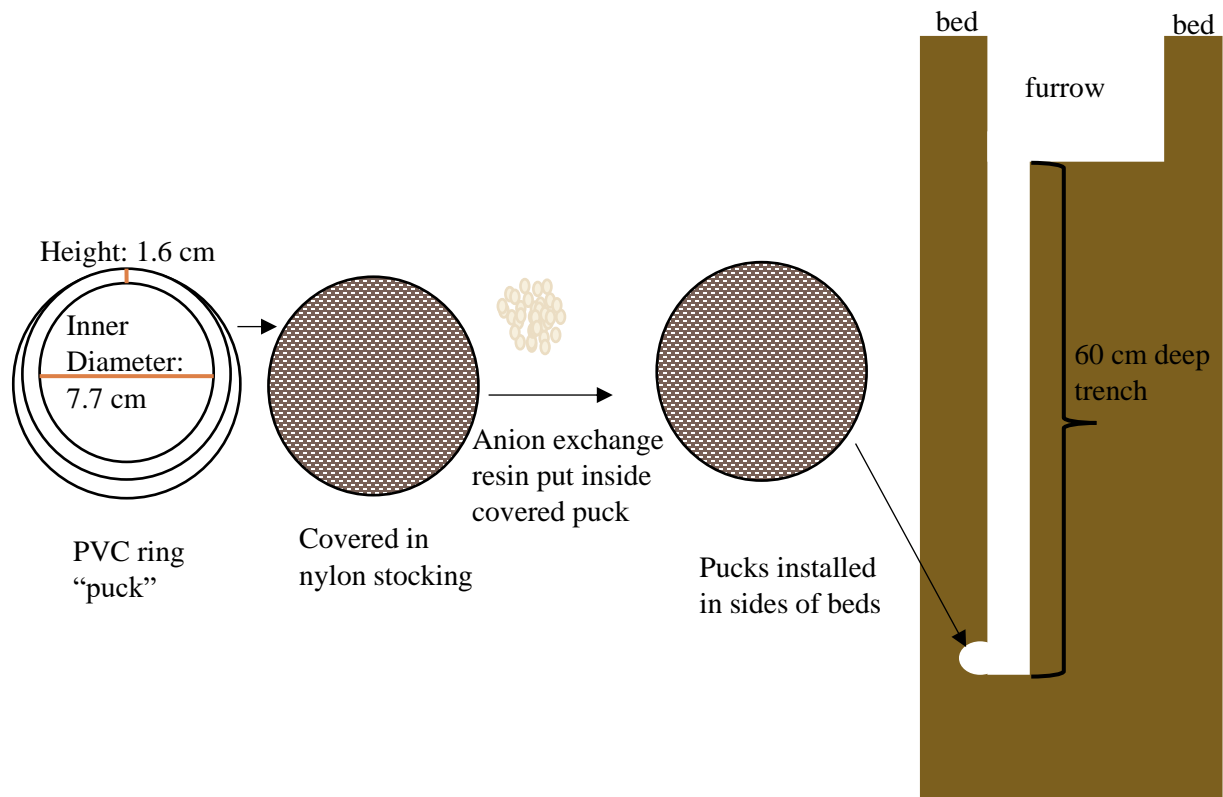
Implementing grazing will not have the same outcomes on soil carbon pools in different environmental conditions and soil types. Depending on the C saturation potential of a soil, driven by the amount of reactive mineral surfaces and environmental conditions like pH and oxygen availability, management practices, like grazing, may need to focus on increasing POM, MAOM or both (Angst et al., 2023), in order to increase total soil C. In our soil, which is high in silt and clay, we would expect there to be a greater capacity to transform and store OM inputs from grazing, like root exudates and manure which have a low C/N ratio, as MAOM over the long term (Angst et al., 2023; Moukanni et al., 2022; Sørensen & Jensen, 1995). This is especially relevant as shifts in aggregation also happen over the longer term.

Animals could also regulate macroaggregate turnover, through hoof action and N inputs which influence microaggregate and mineral associated organic matter (MAOM) formation, both of which are associated with more processed SOM that has the capacity to be stored for a longer time (Lavallee et al., 2020; Six et al., 2004).

10 Conclusion

In integrated crop-livestock systems, animals and crops are produced on the same piece of land, increasing land use efficiency and sustainability. We asked: how does grazing a winter cover crop impact soil health and particularly soil C and N pools in annual, organic vegetable systems in California? We found that the tradeoffs (i.e., compaction, nitrate leaching) associated with grazing cover crops were minimal and animal integration amplified some benefits of leguminous cover crops, particularly timely N provisioning. Grazing increased organic forms of N (i.e., DON, MBN) which is especially important for organic farmers who rely on the soil's capacity to cycle organic nutrients into inorganic forms. Soil pH was lowered by cover crops (both grazed and ungrazed) and soil microbial communities shifted to be more bacteria, specifically gram (+) bacteria, dominated with grazing. It remains unclear how shifts in microbial composition will impact long term outcomes in this system, or if the decrease in POM we observed will eventually lead to greater MAOM storage or simply a decrease in TC. Nevertheless, sheep grazing remains a viable option for organic farmers who are looking to diversify their agroecosystem, increase the biological activity and N cycling in their soil, produce food with fewer external inputs and close the nutrient gaps in our currently disconnected crop and livestock production systems.

11 Supplementary figures



Supplementary Figure 1. Figure and description of resin puck design and installation to measure potentially leachable nitrate (PLN)

- 1) built 144 PVC rings that had a 7.7 cm inner diameter and were 1.6 cm tall, and sanded them to remove abrasive edges
- 2) soaked 144 nylon stockings and pucks in 0.3 M HCl for 10 min, then rinsed each in DI water three times, to remove residual nitrate
- 3) tied the bottom of the stocking and slide the PVC puck into the stocking
- 4) filled the stocking with 50 g of anion exchange resin beads
- 5) tied the open end of the stocking, then doubled it over the puck and tied it again
- 6) completed a full regeneration cycle of the resin beads with a solution of 120 g of NaCl per liter of resin
- 7) rinsed the entire puck with DI 3 times, to remove excess NaCl
- 8) stored pucks a closed container with a small amount of DI to prevent drying out before installation
- 9) dug 60 cm deep trench with trench digger attached to a tractor
- 10) created a ledge at the bottom of the trench and under the bed using a wireless drill gun and installed pucks on the ledge
- 11) attached string and washer to pucks to aid in finding them again
- 12) recorded exact location of every puck by triangulating with permanent objects near the field
- 13) stored blanks at 4 °C for the duration of the puck installation
- 14) returned to exact locations and dug up pucks ~ 11 months later

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