Title

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Publication Date

2023-10-01

DOI

10.1016/j.geoderma.2023.116598

Long-term integrated crop-livestock grazing stimulates soil ecosystem carbon flux, increasing subsoil carbon storage in California perennial agroecosystems

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ABSTRACT

The strategic use of ruminant grazing in perennial cropland is steadily increasing throughout Mediterranean perennial agroecosystems. Integrated sheep-vineyard (ISV) management, where small ruminant livestock graze on understory vegetation, is viewed by some practitioners as a feasible transition opportunity to facilitate less petrochemically intensive vineyard understory management. However, our knowledge of soil carbon dynamics associated with grazing in perennial integrated crop-livestock (ICL) agroecosystems is notably limited, especially within Mediterranean contexts. Here, we use a representative set of on-farm paired surveys to assess soil ecosystem habitat and resource conditions related to SOC flux and storage in vineyards utilizing sheep-integration (ISV) and conventional understory management techniques (CONV). Our results show that long-term grazing increased the quantity of active, labile, and soluble carbon (C) within ISV soils, with much higher quantities of microbial biomass carbon (MBC). Vineyard soils with sheep grazing also showed increases in phospholipid fatty acid (PLFA) biomarkers, particularly amongst core functional groups related to decomposition. Soil microbial communities under ISV had higher C mineralization rates as well as higher carbon use-efficiency, as indicated by less CO_2-C respired relative to the size of the MBC pool. Whereas inorganic soil nitrogen (N) and phosphorous (P) were also higher under ISV, microbial communities showed distinct metabolic investment strategies related to nutrient acquisition, with lower P-cycling enzyme activity and higher N-cycling enzyme activity. Additionally, ISV resulted in an increase in subsoil SOC storage, including higher quantities of physicochemical stabilization in the mineral-associated organic carbon (MAOC) pool of the deepest measured subsoil layer (30–45 cm). We observed no differences in soil structure indicators between treatments nor differences in the carbon fractions associated with four distinct aggregate size categories. We propose a framework to explain observed shifts in SOC dynamics of perennial ICL systems that include i) deposition of C and nutrient inputs with higher lability and solubility; ii) ruminant-induced decoupling of C from N and P, resulting in increased nutrient bioavailability; and iii) altered soil microbial metabolic strategies and more efficient biomass accumulation. These findings show strong potential of strategically applied ICL grazing to enhance soil functioning and increase SOC storage in Mediterranean perennial agroecosystems.

Key words: Soil organic carbon; Soil carbon storage; Grazer-plant-soil interactions; Microbial ecology; Integrated crop-livestock; Perennial agriculture

1. INTRODUCTION

Agricultural resource conservation incentives provide new opportunities to explore underutilized farming methods for their potential agronomic and environmental benefits. One such incentive is to utilize croplands for sequestering additional soil carbon. Increasing the soil organic carbon (SOC) of global croplands has compounding GHG mitigation benefits where it is facilitated by farming methods that rely less on mechanization and heavy use of petrochemical inputs (Minasny et al., 2017). The production of pesticides, synthetic mineral fertilizers, and other inputs produced using large quantities of petroleum and other fossil fuels are well understood to substantially contribute to global GHG emissions (Walling and Vaneeckhaute, 2020; Woods et al., 2010). One historically foundational, yet scientifically understudied management strategy with proposed potential toward these coordinated efforts is the integration of animals and crops within the same production system (Brewer and Gaudin, 2020; Garrett et al., 2017; Russelle et al., 2007). Practitioners of integrated crop-livestock (ICL) management rely on extensively developed local and indigenous knowledge systems, with regional specificity and complexity (Altieri, 1992; Altieri et al., 2012; Garrett et al., 2020; Sekaran et al., 2021b). Whereas much of the world's crop and livestock components are still managed in coordination, with animals providing diverse grazing-based services for crop production, the intensification of U.S. agriculture has resulted in highly specialized and de-coupled farm-scale crop and livestock components (Baur and Iles, 2022; Entz et al., 2005; Garrett et al., 2020; Sanderson et al., 2013).

While more rigorous inquiry is necessary, crop-livestock de-coupling is currently understood to contribute to poor nutrient cycling within and between agricultural operations and an increased environmental footprint of both crop and livestock production (Garrett et al., 2017; Lemaire et al., 2014). As such, the re-integration of crop and livestock system components has been proposed as a strategy toward improving the environmental conservation and resource efficiency outcomes of agricultural landscapes (Russelle et al., 2007). Perennial ICL management, where ruminant livestock forage on understory plant communities during prolonged periods of vegetative growth, provide opportunity as a feasible agroecological alternative to current petrochemically-intensive practices for management of understory plant communities (such as mowing, herbicides, and tillage). Current and developing models of ICL

systems employ diverse, adaptable, and feasible management practices that can be strategically implemented across various scales and crop production systems (Bell et al., 2014; Garrett et al., 2020, 2017; Lemaire et al., 2014). However, the potential of this re-integration, with respect to the comparative benefits and/or trade-offs of this agroecological approach relative to conventional practices, is highly underexplored (Garrett et al., 2017), especially within highly industrialized agricultural contexts (i.e. modern, intensive methods of farming crops and animals for mass production). California, with its extremely diverse agricultural landscapes, provides a unique opportunity for scientific exploration into the potential of this re-integration.

A core proposition of agroecological farming models is to increase the utilization efficiency of externally-applied resources, through retention and (re)cycling processes that are endogenous to the agroecosystem's design and, therefore, *internally regulated* (Altieri et al., 2015; Garcia-Franco et al., 2018; Lipper et al., 2014; Lovell et al., 2010; Snapp, 2017; Wagg et al., 2020). This internal regulation of agroecosystems – defined as its capacity to tightly couple energy and nutrient (re)cycling (King and Hofmockel, 2017; Prommer et al., 2020; Tamburini et al., 2020) – depends upon the dynamic functioning of many complex energy and nutrient transformation processes (Lal, 2016; Power, 2010; Snapp, 2017; Tamburini et al., 2020; Xu et al., 2020). Soils play an essential role in agroecosystem internal regulation – most notably related to the flux of soil carbon (energy) and nutrients, as well as the eventual formation and stabilization of soil organic carbon (SOC).

While not yet explored under integrated crop-livestock (ICL) system contexts, the strategic integration of diverse plant and animal communities across multiple spatial (e.g., field, farm, and landscape) and temporal (e.g., inter- and intra-seasonal) scales have otherwise been shown to promote resource use-efficiency through internal regulation pathways (Altieri et al., 2015; Griesser et al., 2022; Lange et al., 2015; Prommer et al., 2020; Snapp, 2017; Wagg et al., 2020). This may occur through increasing synchronicity in the utilization of carbon (C) and applied nutrients within soil ecosystems (Griesser et al., 2022; Lange et al., 2015; Prommer et al., 2020; Snapp, 2017; Wagg et al., 2020). Some key underlying mechanisms relate to the facilitation of interconnected ecological interactions that include, but are not limited to, niche partitioning and niche complementarity (Altieri et al., 2019; Garland et al., 2020; Petersen-Rockney et al., 2021; Ponisio et al., 2015; Snapp, 2017).

105

106 The impacts of grazing will affect multiple ecosystem processes related to nutrient utilization and the pathways
107 regulating SOC flux and storage (Figure 1) (Brewer and Gaudin, 2020; de Faccio Carvalho et al., 2010; Jarvis, 2009;
108 Lemaire et al., 2014; Rumpel et al., 2015). Specifically, animal re-integration into cropland can alter carbon and
109 nutrient flows *directly* through (1) transformation of aboveground residues into soluble, nutrient-rich, and labile
110 dung and urine, where carbon and nutrients are more stoichiometrically decoupled (Jarvis, 2009; Jung and Allen,
111 1995; Rumpel et al., 2015); (2) biomass removal that triggers shifts in forage productivity and the reallocation of
112 resources above- and belowground (Dawson et al., 2009); and (3) residue deposition and incorporation due to the
113 trampling effect and hoof action of animal traffic (Acosta-Martínez et al., 2004; Greenwood and McKenzie, 2001;
114 Wei et al., 2021) (Figure 1). It may also alter carbon and nutrient flows *indirectly* via (4) inter- and intra-seasonal
115 shifts in plant community composition (Chen et al., 2018) and (5) changes in soil structure, which alter transport and
116 spatial distribution of soil carbon and nutrients as well as their physical protection from continual degradation
117 through occlusion within aggregates (Erktan et al., 2020; Lavallee et al., 2020; Six et al., 2000).

118

119 These modifications to agroecosystem carbon and nutrient inputs have substantial impacts on the size and
120 composition of resource pools and organisms present within cropland soil ecosystems. This may have consequences
121 for microbial ecological processes such as community assembly, substrate utilization and use-efficiency, and
122 microbial energy investment strategies related to resource acquisition, stress responses, and growth rate optimization
123 (Malik et al., 2020). Since the partitioning of SOC into different biochemical and physical pools is mediated by the
124 quantity, quality, and spatial distribution of substrates entering the soil ecosystem (Lavallee et al., 2020; Rasse et al.,
125 2005; Schmidt et al., 2011; Sokol et al., 2019; Sokol and Bradford, 2019), it is likely that the plant-grazer-soil
126 interactions associated with ICL adoption have significant implications for cropland carbon storage dynamics.
127 Ultimately, this partitioning will largely regulate the fate of carbon within soil ecosystems – particularly with respect
128 to how it will be utilized by soil microbes and if it will persist as long-term sequestered C (Cotrufo et al., 2013;
129 Liang et al., 2019; Schmidt et al., 2011; Zhu et al., 2020).

130

The strategic design of ICL systems for improving SOC storage and other conservation outcomes will require improved understanding and consideration of carbon and nutrient flows through the agroecosystem (Brewer and Gaudin, 2020) (Figure 1). Microbial communities largely drive these biogeochemical flows within soils, as they rely on energy from soil carbon decay channels to power nutrient cycling processes (Janzen, 2006; Kopittke et al., 2022; Zhu et al., 2020). Increasing the flux rate of carbon (energy) through soil ecosystems is therefore necessary to increase rates of ecological functioning. Since microbial utilization of SOC for biomass growth (anabolism) is generally associated with respiration and, therefore, CO_2 efflux (catabolism), it has been argued that storing soil carbon is inherently in tension with increasing microbial functioning (Janzen, 2006). However, the accumulation of stable and long-term stabilized mineral-associated organic carbon (MAOC) has increasingly been shown to necessitate the formation of microbial necromass, and is therefore dependent upon the continual pulse and turnover of labile and accessible carbon through the microbial food web (Dynarski et al., 2020; Lehmann and Kleber, 2015; Six et al., 2004, 2002) – a concept known as the microbial carbon pump (MCP). In fact, recent studies have shown that the MCP and its microbially-derived anabolic compounds are the predominate source of stabilized MAOC (Basile-Doelsch et al., 2020; Kallenbach et al., 2016; Liang et al., 2019). Stabilized soil carbon may therefore be viewed predominately as a reservoir of previously processed microbial products, with stored chemical energy which may be accessed later by soil microbial communities when the habitat and resource conditions of the soil ecosystem are altered (Erktan et al., 2020) – a process understood as soil carbon priming (Kuzyakov, 2010). As such, the evaluation and interpretation of soil carbon storage as static and relatively inert *stocks* must be complimented by an understanding of soil carbon *flows* – as flux processes and an energy source for biological functions. These stocks and flows are, of course, related with the production of microbial biomass and accumulation of microbial necromass being critical to building long-term SOC storage.

The goal of this study was to evaluate the soil carbon flux and storage dynamics of perennial integrated crop-livestock systems within the context of working landscapes, with farmer implementation by early adopters who have integrated grazing for multiple years. The use of precision grazing in perennial cropland is steadily increasing throughout California and beyond, particularly within Mediterranean integrated sheep-vineyard (ISV) systems (Ryschawy et al., 2021). However, mechanistic understandings of the legacy effects associated with grazing on

158 nutrient cycling and SOC flux and storage within perennial cropland are lacking. This is especially true in semi-arid
159 regions, which have low precipitation and high temperature features that regulate carbon flows and limit storage
160 pathways (Brewer and Gaudin, 2020; Garcia-Franco et al., 2018; Hoyle et al., 2016, 2013). Conducting on-farm
161 studies across a representative sample of early-adopter ISV practitioners facilitates the important endeavor of
162 evaluating the longer-term impacts of perennial cropland grazing practices within working landscapes (Garrett et al.,
163 2017; Ryschawy et al., 2021) and deepens our understanding of the SOC storage potential associated with perennial
164 crop-livestock re-integration.
165
166 We established an on-farm survey study across a spectrum of ISV early-adoption systems to evaluate the longer-
167 term impacts of perennial cropland grazing practices on SOC fluxes and measurable benefits and/or trade-offs
168 related to soil carbon storage. We explored the hypotheses that sheep grazing of winter soil covers will ***1) increase***
169 ***the quantity of carbon most readily available for processing by the soil food web***; and ***2) increase the flux rate of***
170 ***soil organic carbon turnover***; which should ***3) increase soil organic carbon storage dynamics, especially the***
171 ***fraction stored stably clay mineral surfaces as mineral-associated organic carbon (MAOC).*** The objectives of this
172 study were to better understand cropland grazing impacts on SOC and biogeochemical processes for the purpose of
173 developing best management practices (Garrett et al., 2017; Niles et al., 2018), informing adoption through
174 identification of potential benefits and tradeoffs, and improving our understanding of the climate change mitigation
175 and soil C sequestration potential of perennial cropland grazing (Brewer and Gaudin, 2020).
176
177 **2. MATERIALS AND METHODS**
178 ***Ethics statement***
179 Permission for site access was previously granted by landowners. All sites were privately owned and no permits
180 were required.
181
182 ***2.1 Study region and management characteristics***
183 A soil survey of paired vineyard sites was conducted in the Northern California coastal foothills at three locations in
184 2018 and one location in 2021, between the months of January and March (Figure 2). The paired sites sampled in

2021 were added to strengthen and validate the initial findings from the 2018 sampling. Paired vineyard sites at each sampling location consisted of one '*non-integrated*' vineyard (interrow vegetation managed through mowing; CONV) and one adjacent '*integrated*' vineyard (interrow vegetation managed through grazing for 10+ years; ISV) – with one location in Sonoma County (home to Wappo and Patwin native peoples), two in Lake County (home to Pomo, Lake Miwok, and Patwin native peoples), and one in southern Mendocino County (home to Pomo and Yuki native peoples) (8 paired vineyards across 4 locations) (Figure 2). This Mediterranean climate is classified as a semi-arid Köppen-type *Csc* (Beck et al., 2018). It is characterized by mild cool winters, warm and dry summers, and seasonal mean annual precipitation that is lower than the regional evapotranspiration (ET) potential. The annual regional precipitation for sites in 2017 and 2020 was 739 mm and 368 mm, respectively. The mean maximum and minimum temperatures were 21.9°C and 5.7°C in 2017 and 25.8°C and 5.3°C in 2020, respectively. This contributed to an annual potential evapotranspiration (ET_o) of 1145 mm (2017) and 1406 mm (2020) and, therefore, a 1.5x (2017) and 3.8x (2020) higher potential water demand than the regional precipitation supply. As was the case for all vineyard sites within this study, the vast majority of Northern California regional vineyard systems utilize micro-irrigation, especially surface drip systems (Tindula et al., 2013), to match vine ET demand during the warm, dry vine growing season (Prichard, 2000). This growing season generally begins in March (bud break) and goes throughout August to October (harvest), depending on the winegrape varietal. Irrigation was not applied to the interrow space, where ISV grazing predominately occurs (Niles et al., 2018; Ryschawy et al., 2021). The understory vegetation growing season is instead limited to periods of sustained precipitation, which typically occurs between November and April, during which 91% of all regional rainfall has occurred over the last 20 years (http://www.cimis.water.ca.gov/). This is also the period in which the vast majority of sheep grazing in regional vineyards occurs, including the ISV sites utilized within this study.

Most typically, sheep-vineyard grazing within this region is used as an understory plant growth termination methodology, similarly to the application of mowing, and is most often implemented immediately before vine bud break (Niles et al., 2018; Ryschawy et al., 2021). Though less common, sheep grazing sometimes occurs multiple times across the understory growing (vine dormancy) and vine growing seasons, where it is strategically applied to achieve additional management benefits such as vine leaf thinning and removal of suckering trunks (Niles et al.,

212 2018; Ryschawy et al., 2021). The grazing strategies on all four of the integrated vineyards in this study were
213 characterized as *high-density, short-duration rotational grazing* management (de Faccio Carvalho et al., 2010). This
214 rotational grazing strategy incorporate small paddocks that are grazed with high animal density and rotated
215 frequently amongst larger sections of the overall landscape. This strategy facilitates longer rest periods and
216 increased competition amongst grazing ruminants (Teague et al., 2008). This has been found to lower the duration of
217 grazing per unit of land area and reduce grazing selectivity and the spatial heterogeneity of grazing pressure (Teague
218 et al., 2008; Teague and Dowhower, 2003). Briefly described, temporary electrical fencing was erected to establish
219 1-acre sized grazing paddocks, where ~250 ewes were grazed for 1-2 days within each paddock before rotating to
220 the next temporary paddock. Grazing generally occurred once during vine dormancy. The timing of grazing events
221 varied with precipitation and understory plant growth rates, but generally occurred sometime between early March
222 through late April, before vine bud break and a coinciding decrease in regional precipitation rates. Each grazing
223 event aims to remove roughly 80% of understory biomass from the vineyard as a seasonal "termination" of the
224 vineyard understory plant community, which remained dormant throughout the warm, dry vineyard growing season.
225 Grazing sometimes occurred two or more times during the dormant season (November to April), when forage
226 productivity was substantially high. Before transitioning to sheep grazing of understory vegetation, sites were
227 managed by a mixture of mowing and herbicides, which generally occurred at the same time of year as grazing. The
228 undervine row of all four mowed (CONV) vineyards and one grazed vineyard (ISV; Site 3) were managed using
229 synthetic herbicide applications (Table 1). Both the ISV and CONV vineyards at Site 1 used conservation tillage,
230 with shallow (<10 cm depth) tillage of every other row in alternating years (Table 1).
231
232 ***2.2 Site selection and participatory engagement***
233 Study sites were first selected based on identifying early adopters of ISV management – vineyards that had a long-
234 term legacy of grazing sheep – and selection of participating vineyards was confined to the Northern California
235 coastal foothill region to reduce climate and soil variability (Table 1). While perceptions of ISV management are
236 increasingly favorable among adopters and non-adopters alike (Ryschawy et al., 2021), vineyard grazing is still
237 considered a niche production system compared to the dominant technological regimes (Garrett et al., 2020;
238 Ryschawy et al., 2021) and early adopters of ISV practices in California are rare. Producers utilizing ISV

management were identified using directories from the *LandSmart* collaborative (http://landsmart.org) and grower networks from the Community Alliance with Family Farmers (CAFF). Four integrated vineyards (ISV) were selected based on grower knowledge of long-term sheep grazing and co-management legacy. These ISV growers expressed interest in participating and worked with the study's authors to identify the adjacent non-integrated vineyards (CONV) for comparison. A participatory survey was conducted to collect management information for each vineyard (vine/rootstock varietal, understory vegetation management, external amendments, irrigation, and tillage) to assess and minimize variability between paired sites (Table 1). Soil type and topography data was collected from the USDA-NRCS *SoilWeb* app (https://casoilresource.lawr.ucdavis.edu). Regional soils are Inceptisols (USDA-NRCS), with textures ranging from clay loam to loam and an average clay content of 27%±2% (Table 1). At the time of the study, two of the ISV vineyards (Site 1 & 4) were organic certified through the California Certified Organic Farmers (CCOF) program.

Sampling plots (2 ha area) within each paired integrated (ISV) and non-integrated (CONV) vineyard site were selected to maximize the edaphic and co-management similarity of paired sites and isolate the impact of sheep grazing. Wine vineyards provide a unique opportunity for soil surveying, in that tightly controlling management variability, especially related to water and soil fertility, is essential for improving wine grape quality. Vineyard growers strategically limit irrigation and N uptake at certain stages of vine phenology to control vegetative growth and mitigate various perceived tradeoffs between vine vigor and wine grape quality (Spayd et al., 1994; Wheeler and Pickering, 2003; White et al., 2007). As such, excessive water and N availability is generally avoided by reducing inputs (Gaiotti et al., 2017; Lazcano et al., 2020). When inputs are utilized they are most often applied in small doses, timed only when vine demand is highest, and delivered directly under the vine (Peter Christensen et al., 1994; Spayd et al., 1994). Consequently, while cover crops are increasingly utilized to prevent soil erosion and stabilize soil quality (Novara et al., 2019; Rodrigo-Comino, 2018), wine vineyards are otherwise often low-input agroecosystems, especially within the vineyard interrow, and thus have less co-management variability than other perennial agroecosystems.

2.3 Soil collection and processing

Soil sampling occurred once per vineyard site and was timed before the seasonal understory forage termination event(s) to maximize the soil acclimation period between the last graze or mow events. Soil samples were collected from eight randomly selected points per 2 ha plot in a "W" pattern (Moebius-Clune et al., 2016). Sub-plots (1 m^2) were set-up at each sampling point, surface residues were removed, and three soil cores (5 cm diameter) were taken at three depths (0–15 cm, 15–30 cm, and 30–45 cm) in the vineyard interrow. Samples were not taken from the undervine row to minimize management variability due to seasonal irrigation, fertility, and herbicide applications. Samples were weighed in the field, homogenized and composited for each sub-plot, and placed in a cooler for transport. Bulk soils were processed promptly and stored at 4 °C until further analysis, except for ~75 g of soil that was separated and stored at −80°C for PLFA and enzyme assays. Approximately 10 g of field moist soil was sieved (2 mm) and oven dried (105°C) to a constant weight to determine soil gravimetric water content (GWC). Surface soil (0–15 cm) bulk density (BD) was determined for each soil core using mass of oven-dried soil (105 ˚C, 24 h or until consistent weight) and total volume of each soil core (Blake and Hartge, 1986). Another 250 g of soil was subsampled for chemical analysis and ~100 g was used to determine texture and soil aggregate characteristics.

2.4 Soil chemical properties

A subsample of ~300 g was sent to a certified laboratory (Ward Laboratories – Kearney, NE) for analyses of soil texture (sand:silt:clay) by hydrometer; pH (1:1 v/v method); soil salinity by electrical conductivity (EC; dS/m); available P (mg kg^{-1}) via Olsen bicarbonate extraction; and cation exchange capacity (CEC) (Meq 100 g^{-1}) based on ammonium acetate extraction and pH. All soil depth fractions were dried to constant mass, ball-milled, and weighed for total elemental C and N using dry combustion (Costech ESC 4010 Elemental Analyzer – Valencia, CA, USA). Soil NH_4^+ and NO_3^- were extracted from 5 g of fresh soil with 20 ml 2 M KCl solution and measured using colorimetric assays on a BioTek Synergy HTX (BioSPX B.V. – The Netherlands) microplate reader. Mineral nitrogen is the sum of soil NH_4^+ and NO_3^-. Soil organic carbon (SOC) and nitrogen (SON) were measured by subtracting HCl inorganic C extraction measurements and inorganic N pools (NO_3^- and NH_4^+) from total elemental C and N analyses described above.

2.5 Soil aggregate size distribution and aggregate-associated carbon

Aggregate size categorization was performed on air-dried soils by wet sieving to separate water-stable aggregates into four size categories: large macro-aggregates (2000 µm), small macro-aggregates (250-2000 µm), micro-aggregates (530-250 µm), and the silt and clay fraction (<53 µm) (Cambardella and Elliott, 1993; Kemper and Rosenau, 1986). Each soil was submerged in deionized water for 10 minutes before wet-sieving, and a sub-sample was taken to assess soil gravimetric water content (g g^{-1}) after saturation. A 40 g sub-sample of saturated soil was then transferred to a vibratory sieving tower with rainfall simulator (Fritsch Analysette 3 Pro – Idar-Oberstein, Germany), with vibration amplitude set at 0.1 µm and frequency at 50 Hz. Sieving lasted until the deionized water used to wash soils on the sieve was flowing clear, which was generally around 60 seconds. The remaining fractions on each sieve (2000, 250, and 53 µm), as well as the soil-water suspension passed through the 53 µm sieve (<53 µm), were dried at 60° C to dry until reaching constant weight. The mass recovery threshold was set between 0.98 – 1.02% and was calculated as follows **(1)**:

$$(1)\ Mass\ recovery\,(\%) = bulk\ soil\,(g) - ¿¿$$

where bulk soil (g) is the mass of the soil used for wet-sieving of each sample; GWC (g g^{-1}) is the gravimetric water content of the soil used for wet-sieving; and A_i is the oven-dry weight (g) of each aggregate fraction. When samples did not meet the mass recovery threshold, the samples were repeated. Mean weight diameter (MWD), a weighted-average index of aggregate stability (van Bavel, 1950), was calculated as follows **(2)**:

$$(2)\ MWD = \sum_{i=4}^{4} \acute{X}_i \cdot A_i$$

where X_i is the average diameter (µm) for particles of each *i*-level aggregate fraction and A_i is the weight percentage of the fraction in the bulk soil. SOC content was measured for each aggregate fraction using combustion analysis (Costech ESC 4010 Elemental Analyzer – Valencia, CA, USA). The proportional concentration of SOC in each aggregate fraction was calculated as follows **(3)**:

$$(3) M_i = \frac{A_i \cdot SOC_i}{\sum_{i=1}^{4} A_i \cdot SOC_i}$$

where M_i is the relative SOC concentration of each *i*-level aggregate fraction (%); A_i is the oven-dry weight (g) of each aggregate fraction; and SOC_i is the relative SOC concentration of each *i*-level aggregate fraction. The SOC stock for each aggregate fraction was calculated as follows **(4)**:

$$(4) M_i = C_i \cdot SOC_i \cdot BD \cdot H \cdot 10^{-1}$$

where M_i is the SOC stock of each *i*-level aggregate fraction (t hm^{-2}); C_i and SOC_i are the relative fraction and SOC concentration each of *i*-level aggregate fraction, respectively; BD is the soil bulk density (g cm^{-3}) and H is the thickness of soil layer, which was 15 cm for this measurement.

2.6 Soil organic carbon size fractionation

Soil organic carbon was separated into particulate organic carbon (POC), and mineral-associated organic carbon (MAOC) using aggregate dispersion, wet sieving, and particle-size fractionation method (Six et al., 1998). In short, 20 g of air-dried soil was dispersed with 100 ml of 5% (w/v) sodium hexametaphosphate ($Na_6(PO_3)_6$) and an 18-hour rotary shaking for sufficient dispersion. Dispersed soils were washed through a 53 µm sieve on a vibratory sieve shaker (Fritsch Analysette 3 Pro – Idar-Oberstein, Germany) as described in Section 2.5. The fraction retained on the sieve was considered as POC, while the finer fraction that passed through the sieve was considered as MAOC. Both POC and MAOC fractions were dried at 60° C until reaching constant weight, then ground, and analyzed for total C on an elemental analyzer (Costech ESC 4010 Elemental Analyzer – Valencia, CA, USA). The final content of POC or MAOC in bulk soil was calculated based on the recovered mass. For example, POC (mg C g^{-1} bulk soil) was calculated with two formulas as follows **(5)** and **(6)**:

$$(5)\ Mass\,recovery\,(\%) = [(POC\,(g) + MAOC\,(g))\,/\,bulk\,soil\,(g)] \times 100$$

$$(6)\ POC\,(mg\,C\,g^{-1}\,bulk\,soil) = \frac{POC\,(g) \cdot POC\,(mg\,C\,g^{-1})}{bulk\,soil\,(g) \cdot (mass\,recovery \cdot 0.01)}$$

where bulk soil (g) is the mass of the soil used for wet-sieving of each sample; POC and MAOC (g) are the masses of the POC and MAOC fractions recovered after the wet-sieving, respectively; and POC (mg C g^{-1}) is the C concentration measured in the POC fraction.

2.7 Soil microbial biomass and dissolved organic carbon

Soil microbial biomass carbon (MBC) was measured using the fumigation-extraction method (Horwath and Paul, 1994). Fresh soil was sieved to 4 mm and two replicates of 6 g were weighed into glass vials. One replicate was fumigated for 24 h with chloroform ($CHCl_3$) and the other sample (unfumigated) was immediately extracted using 30 ml 0.5 M K_2SO_4. After chloroform fumigation, the fumigated sample was also extracted using 30 ml 0.5 M K_2SO_4. The extracted solutions were filtered with Q5 filter paper and then analyzed for TOC and TN (Elementar TOC/TNb – Ronkonkoma, NY, USA). Microbial biomass C was calculated as the difference in dissolved C concentration between fumigated and unfumigated samples, using a K_e conversion factor of 0.35. The dissolved C content in the unfumigated samples represent the dissolved organic C (DOC) fraction (Jones and Willett, 2006). The microbial quotient (Q_{mic}) represents the ratio of MBC relative to SOC (ug MBC/ug SOC) (Sun et al., 2020).

2.8 Carbon mineralization

A 35-day (Haney et al., 2008) incubation was conducted to determine C mineralization over time, as well as potentially mineralizable C (PMC; the flush of CO_2 during a 72-hour incubation (Wade et al., 2018)) using rewetted air-dried soils. Three technical replicates were run per sample to account for potential methodological variability (Wade et al., 2018). Briefly, 15g of air-dried soil (0-15 cm depth) was sieved (4 mm) and weighed into 50-ml glass beakers. Each sample was rewetted from above to 50% water-filled pore space and placed into 1-quart mason jars. Each sample was placed inside a 0.4 L mason jar, capped with a metal lid and a rubber septum, and incubated at 25

°C for 35 days. Respired CO_2 was determined by sampling the headspace gas using a continuous-flow CO_2/H_2O gas analyzer (LI850 – LI-COR Biosciences, Lincoln, NE, USA) at 1, 3, 7, 14, 21, 28, and 35 days. Jars were opened every 7 days to equilibrate with the atmosphere and allow replenishment of oxygen, but otherwise remained sealed with no air flow. Soil water loss from evaporation was low. The soil evaporation was replaced on days 14 and 21 by re-weighing soils and adding deionized water accordingly. Respiration for each sampling date was calculated as the difference between a sample and a control, using the ideal gas law and adjusting for the total headspace. Net respiration was calculated as the sum of the respiration measurements up to each sampling date. Soil C mineralization was expressed both in terms of cumulative C mineralization ($Cmin_{total}$; ug CO_2-C g $soil^{-1}$ day^{-1}) (Grunwald et al., 2016) and relative to the SOC content of each sample ($Cmin_{soc}$; mg CO_2-C g SOC^{-1} day^{-1}) (Zhang et al., 2007). The metabolic quotient (Q_{met}) represents the ratio of microbial respiration to microbial biomass and was determined by dividing the 24-hour basal respiration (M_{resp}; ug CO_2-C g^{-1} dry soil) by MBC (ug MBC g^{-1} dry soil) (Anderson and Domsch, 1993).

2.9 Microbial community structure and exocellular soil enzymes

Soil microbial community structure was characterized using phospholipid fatty acid (PLFA) analysis (Ward Laboratories – Kearney, NE) using a chloroform-methanol extraction and gas chromatograph with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column (Bossio and Scow, 1998). Community structure bioindicators for PLFA were distinguished into bacterial groups including Gram-positive (Gram(+)) and Gram-negative (Gram(-)) bacteria, actinomycetes, and fungal groups including saprophytic fungi and arbuscular mycorrhizal fungi (AMF). The Gram(+)/Gram(-) and fungal/bacterial (F/B) ratios represent the relative distribution of Gram(+)-to-Gram(-) bacterial biomass and fungal-to-bacterial biomass, respectively.

Exocellular enzyme potentials included BG: β-Glucosidase (glycoside hydrolysis), CB: β-D-cellubiosidase (cellulose decomposition), LAP: L-aminopeptidase (peptide hydrolysis), and PHOS: Alkaline-Phosphatase (phosphate hydrolysis) were measured using fluorescence microplate assays (Bell et al., 2013). Briefly, 2.75g of soil was blended with 91 ml of 50 mM sodium acetate buffer and pH adjusted to the average pH of soil samples from a given vineyard. The soil slurry was then mixed on a stir plate as 800 µl were transferred into a deep 96-well plates.

Substrate concentrations and incubation time were determined based on calibration tests to capture the maximum potential enzyme activity. 600 µM of fluorescently labeled substrates were added for all enzymes assayed, except LAP, where 400µM were added. A 200 µL aliquot of substrate was pipetted into the sample and incubated for 3 h at 25°C. Standard curves were prepared for each sample using 4-methylumbelliferone or 7-amino-4-methylcoumarin for LAP. After incubation, assays were centrifuged for 3 min at 1500 rpm and 250 µL of supernatant was pipetted into black 96-well plates. Substrate fluorescence was measured on a BioTek Synergy HTX microplate reader (BioSPX B.V. – The Netherlands) at wavelengths 365 nm (excitation) and 450 nm (emission). Urease (urea hydrolysis) was measured using a standard colorimetric assay method (Kandeler and Gerber, 1988). The enzyme activity was calculated based on the soil dry weight and incubation time (unit: nmol g^{-1} h^{-1}).

2.10Statistical analysis and mixed model selection

Statistical analyses were conducted using the statistical software R, version 3.6.3 (Team, 2021) Linear mixed-effect regression models were used to measure univariate treatment effects across all vineyard pairs, as well as difference between treatments within each vineyard pair. Models were fit using fixed effects for 'treatment' (ISV vs. CONV) and 'location' as well as their interaction term (treatment(*x*)location) to estimate differing treatment effects on response variables across locations with the *lmer* and *lmerTest* packages (Bates et al., 2015; Kuznetsova et al., 2017). We accounted for our sampling design by using a nested 'plot' (vineyard sampling zone nested within location) as a random effect, which yielded the lowest Akaike information criterion (AIC) score when included. Depth was not included as a factor in the model. Instead, each depth was analyzed independently using the model described above. Given the degree of multiple comparison testing associated with this univariate approach, MANOVA was conducted for soil carbon response variables as a false discovery rate (FDR) controlling approach using the *dplyr* package, in order to correct for random events that falsely appear significant as revealed by our univariate assessments (Benjamini and Hochberg, 1995). Before conducting MANOVA, the *mvnormtest* package was used to conduct a Shapiro-Wilk test for multivariate normality and the absence of multicollinearity was checked by conducting correlations among the response variables, which all measured $R^2 \leq 0.80$ and therefore presented no concern. Our MANOVA revealed similar patterns in soil carbon response variables to our univariate approach.

We further tested factors associated with the climatic and edaphic differences (i.e., MAP, MAT, %clay) among vineyards as covariates. These covariates were left out from final models as none of the environmental factors were significant or strongly influenced our main model effects. Residuals were checked for normality and homogeneity of variance. When variables were non-normally distributed or had unequal variance, data were log or square root transformed prior to calculation of means and back-transformed for visualization. Fixed effects were investigated with means comparisons and considered p-value < 0.001(***) as highly significant, p-value < 0.01(**) as significant, and p-value < 0.05(*) as marginally significant. Non-significant means comparisons with p-value < 0.10 were considered as a *'trend'* (Hurlbert and Lombardi, 2009; Wasserstein et al., 2019). Tukey's pairwise comparisons were used to assess differences between each treatment within each of the four study locations. Values in tables and graphs are reported as comparisons within each site, whereas values reported in the results section are averages across sites. Box plots were graphed using the *ggplot2* package in R. The horizontal line is the mean, and upper and lower sectors are the first and third quartiles, respectively. Upper and lower 'whiskers' extend to the highest or lowest value, respectively, within 1.5 times the inter-quartile range (the distance between the first and third quartiles).

3. RESULTS

3.1 Soil physicochemical habitat

Management treatment (ISV vs. CONV) had a significant effect on several key soil physicochemical indicators (Table 2). On average across all vineyards, the ISV treatment increased dissolved P (19.8±1.7 vs. 11.5±1.3 ug g^{-1}; p<0.001), Total N (TN;2.1±0.08 vs. 1.6±0.06 g kg^{-1}; p=0.013), and salt content (EC; 0.21±0.02 vs. 0.12±0.006 dS m^{-1}; p=0.058) in surface soils (0–15 cm) compared to CONV management. The increase in P was significant in all four paired vineyards and EC values were significantly higher in three out of four of the paired vineyard surface soils. While dissolved P, TN, and EC were not significantly different in the subsoil (15–30 and 30–45 cm), the mineral N fraction (NH_4^+ + NO_3^-) was significantly higher under ISV management at the 30–45 cm depth (5.1±0.4 vs. 3.3±0.3; p=0.042). While mineral N values were higher under ISV in the 0–15 cm (14.1±1.2 vs. 8.6±0.8; p=0.183) and 15–30 cm (6.3±0.5 vs. 4.1±0.3; p=0.211) depths, these increases were non-significant. Grazing did not

significantly affect soil pH or CEC at any depth zone, although treatment effects for CEC varied between locations (treatment(*x*)location p=0.041).

The physical characteristics of surface soils (0–15 cm) as indicated by compaction (BD; 1.32±0.03 vs. 1.37±0.03 g cm^{-3}; p=0.634; Table 2) and aggregate stability (MWD; 1.47±0.13 vs. 1.44±0.12; p=0.953; Supplementary Figure 1) were not affected by management across any of the four locations. There was also no difference in the relative size distribution of surface soil (0–15 cm) aggregates (Supplementary Figure 1) between ISV and CONV treatments for *macroaggregates* (>2000 um; 21.2±2.5% vs. 19.9±2.3%; p=0.959), *large microaggregates* (250–2000 um; 32.6±2.4% vs. 36.4±2.7%; p=0.791), *small microaggregates* (53–250 um; 22.5±1.9% vs. 20.2±1.6%; p=0.835), or the *silt and clay* fraction (<53 um; 23.7±2.3% vs. 23.5±2.4%; p=0.635). Soil water content was also similar in both treatments at all depth zones: 0–15 cm (0.21±0.01 vs. 0.18±0.1 g g^{-1}; p=0.798), 15–30 cm (0.21±0.01 vs. 0.19±0.01 g g^{-1}; p=0.777), and 30–45 cm (0.22±0.01 vs. 0.23±0.01 g g^{-1}; p=0.851).

3.2 Soil microbial community structure and enzymatic activity

The surface soil (0–15 cm) abundance (ng g^{-1}) of total phospholipid fatty acid (PLFA) biomarkers, an indicator of viable microbial biomass, significantly responded to long-term grazing (Table 3; p<0.001). Individual vineyard pairs ranged from 24.6% to 64.9% higher total PLFA abundance under ISV (3543±304) than CONV (2543±234) management, with significantly higher total PLFA abundance in three out four paired vineyards. Structural biomarkers showed higher abundance of both bacterial (1497±149 vs. 956±102; p<0.001) and fungal (385±49 vs. 263±33; p=0.004) groups in ISV vineyards across sites. Mole percent distribution (mol%) also indicated an increase in bacteria abundance (38.9±1.8% vs. 34.5±1.6%; p<0.001), but no changes for fungi under long-term grazing (9.1±1.0% vs. 9.1±1.2%; p=0.991). While total bacterial biomarkers increased under ISV in three out of four paired vineyards, total fungal biomarkers treatment response did not differ across locations (Table 3), despite a significant main treatment effect. One location (Site 4) showed very low total fungal biomarker abundance values in both the ISV (7±6 ng g^{-1}) and CONV (12±9 ng g^{-1}) treatments. The relative abundance of bacterial and fungal groups was not affected by grazing (F/B; 0.22±0.02 vs. 0.22±0.02; p=0.887).

The main treatment effect varied for specific fungal and bacterial functional groups, with higher abundances under ISV for saprophytic fungi (261±35 vs. 170±23; p=0.004) and actinomycete (232±21 vs. 141±16; p<0.001), but no significant difference between ISV and CONV for arbuscular mycorrhizal (AM) fungi (124±15 vs. 91±17; p=0.544). There was also no significant main treatment effect between ISV and CONV for Gram(+)/Gram(-) ratio (1.49±0.13 vs. 1.37±0.10; p=0.384) and saturated/unsaturated fatty acid ratio (3.93±0.79 vs. 3.85±0.72; p=0.909), although the saturated-to-unsaturated fatty acid ratio treatment(x)location interaction (p=0.021) indicated variability in the treatment response between paired vineyards.

The surface soil (0-15 cm) exocellular enzymatic activity potentials (nmols g OD soil^{-1} hour^{-1}; Figure 3) related to nitrogen cycling were higher under ISV compared to CONV management for both L-aminopeptidase (peptide hydrolysis) (30.9±2.6 vs. 21.1±2.1; p<0.001; Figure 3A) and Urease (urea hydrolysis) (26.6±1.8 vs. 14.7±1.2; p=0.001; Figure 3B). Pairwise comparisons for Urease activity showed significant treatment effects at three out of four paired vineyards, while L-aminopeptidase was higher in the ISV treatment at only one paired vineyard. Phosphatase (phosphate hydrolysis) was lower under ISV management compared to CONV (79.2±8.1 vs. 94.9±8.8; p=0.017; Figure 3C), with significant effects at only one site. There was no significant treatment effect for enzymes related to carbon cycling – β-Glucosidase (glycoside hydrolysis) (54.1±6.1 vs. 38.0±4.8; p=0.524; Figure 3D) and β-D-cellubiosidase (cellulose decomposition) (10.3±1.2 vs. 9.8±1.2; p=0.639; Figure 3E).

3.3 Soil carbon flux pools and metabolic activity indicators

Soil carbon flux pools, indicative of labile and active soil carbon (Figure 1), were strongly impacted by animal grazing. Although soil carbon flux pool values were generally highest in surface soils (0–15 cm) across both treatments, ISV management was most significantly impactful in the subsoil (Figure 4). Microbial biomass C (MBC; ug g^{-1}) was significantly higher under ISV management compared to CONV at all depth zones: 0–15 cm (454±30 vs. 245±22; p=0.050; Figure 4A), 15–30 cm (174±15 vs. 94±9; p=0.008; Figure 4B), and 30–45 cm (150±12 vs. 70±7; p<0.001; Figure 4C). While there was no significant main treatment variation in dissolved organic C (DOC; ug g^{-1}) or potentially mineralizable C (PMC; ug g^{-1}) at all depths, DOC contents trended higher in the 30–45 cm depth of

the ISV treatment (98±5 vs. 76±5; p=0.118; Figure 4F). PMC also trended higher at both the 15–30 cm (10.9±0.8 vs. 6.7±0.3; p=0.064) and 30–45 cm (5.5±0.3 vs. 4.4±0.3; p=0.081) depths (Figure 4H-I).

The soil microbial quotient (Q_{mic}; MBC:SOC) was significantly higher in ISV surface soils (0–15 cm) compared to CONV (0.018±0.001 vs. 0.012±0.001; p=0.034; Figure 5A). The Q_{mic} main treatment effect was non-significant in both subsoil depths: 15–30 cm (0.016±0.002 vs. 0.011±0.001; p=0.241; Figure 5B) and 30–45 cm (0.024±0.003 vs. 0.016±0.002; p=0.269; Figure 5C). The soil metabolic quotient (Q_{met}; M_{resp}:MBC) was also impacted by long-term grazing similarly across sites. ISV management significantly lowered Q_{met} values compared to CONV in 0–15 cm surface soils (0.023±0.003 vs. 0.056±0.019; p=0.049; Figure 5D). While Q_{met} values also trended lower in the 30–45 cm subsoil depth of ISV vineyards (0.021±0.003 vs. 0.036±0.005; p=0.104), Q_{met} was not significantly affected by treatment in the 15–30 cm depth 0.038±0.020 vs. 0.44±0.006; p=0.240) (Figure 5E-F). Cumulative soil carbon mineralization ($Cmin_{total}$) rates, measured via respiration over a 35-day incubation period, showed similar trends between ISV and CONV in surface soils (0–15 cm) with a cumulative rate of 1.28±0.11 vs. 1.09±0.12 ug CO_2-C g $soil^{-1}$ day^{-1}, respectively (p=0.853; Figure 6A). The $Cmin_{total}$ rate was significantly higher under ISV in the 15–30 cm subsoil depth (1.89±0.29 vs. 1.46±0.18 ug CO_2-C g $soil^{-1}$ day^{-1}; p=0.048; Figure 6B), though not significantly different at 30–45 cm (0.37±0.04 vs. 0.33±0.03 ug CO_2-C g $soil^{-1}$ day^{-1}; p=0.397; Figure 6C).

3.4 Soil carbon stabilization and storage pools

Total SOC (g kg^{-1}) was highest in the surface soils (0–15 cm) and decreased in total quantity with increasing depth in both ISV and CONV treatments, (Figure 7A-C). The main treatment effect on soil carbon storage pools was generally most significantly impactful in the subsoil (15–30 and 30–45 cm) and we observed no significant differences in total SOC content of surface soils (0–15 cm; 26.1±1.2 vs. 21.4±0.9; p=0.197; Figure 7A). Within subsoils, SOC content trended higher in the 15–30 cm depth (12.4±0.6 vs. 8.9±0.5; p=0.063) and was significantly higher at 30–45 cm (8.3±0.6 vs. 6.2±0.6; p=0.003) under ISV management (Figure 7B-C). Similarly, the main treatment effect for MAOC was not significant between ISV and CONV treatments at both 0–15 cm (15.8±0.6 vs. 13.3±0.6; p=0.168; Figure 7D) and the 15–30 cm subsoil depth (11.0±0.5 vs. 8.6±0.5; p=0.185; Figure 7E), but did MAOC significantly increased under ISV compared to CONV management in the 30–45 cm subsoil depth (8.1±0.6

vs. 5.9±0.5; $p<0.001$; Figure 7F). The POC fraction showed opposite trends with a significant main treatment effect at 0–15 cm and higher surface soil POC under CONV management (3.5±0.3 vs. 6.2±0.5; $p=0.012$; Figure 7G), but no significant effects in either the 15–30 (1.0±0.1 vs. 1.0±0.2; $p=0.620$) or 30–45 cm (0.4±0.04 vs. 0.5±0.06; $p=0.472$) subsoil (Figure 7H-I).

There was no impact of long-term ISV grazing on absolute SOC values (g kg^{-1}) in surface soils (0–15 cm) for the macroaggregate (>2 mm; 6.7±0.9 vs. 5.7±0.8; $p=0.964$; Figure 8A), large microaggregate (250–2000 um; 8.6±0.7 vs. 8.6±0.7; $p=0.706$; Figure 8B), and silt and clay fractions (<53 um; 5.4±0.4 vs. 4.2±0.3; $p=0.219$; Figure D) across sites. The ISV treatment did trend higher than CONV for the aggregate-associated C content within small microaggregates (53–250 um; 5.1±0.5 vs. 3.4±0.3; $p=0.107$; Figure 8C). Management treatment also did not shift the relative distribution (% of total C; Supplemental Figure 2) of surface soil (0–15 cm) SOC across *macroaggregates* (>2000 um; 25.3±2.9% vs. 21.8±2.2%; $p=0.447$), *large microaggregates* (250–2000 um; 31±2.4% vs. 39.7±2.1%; $p=0.621$), *small microaggregates* (53–250 um; 20.9±2.1% vs. 15.9±1.2%; $p=0.149$), and the *silt and clay* fraction (<53 um; 22.6±2.3% vs. 22.6±2.6%; $p=0.871$).

4. DISCUSSION

Conducting on-farm studies with early-adopter ISV practitioners facilitates the important endeavor of evaluating the impacts of perennial cropland grazing practices within the context of their on-the-ground application. In lieu of developing extensive perennial ICL monitoring trials, survey studies provide our best opportunities to explore long-term comparisons of these systems characteristics and potential. However, an important context consideration when interpreting these results is the limitation in study sites and variation in characteristics between sites (Table 2). Soil texture and clay content (%clay) – important parameters related to soil carbon cycling – were relatively similar amongst sites. However, Sites 2 and 3 occurred on sloping hills (15-30% slopes) relative to the flat bottomlands at Sites 1 and 4. The duration under current management practices was heterogeneous across sites, largely due to more variation in the length of CONV managed vineyards. The ISV vineyards had relatively similar management durations, providing comparable periods of acclimation to grazing across sites.

557 Climatic and management characteristics were qualitatively similar amongst sites. Most notably, the vineyard
558 interrow (where grazing and soil sampling both occurred) received no external water or nutrient applications at any
559 site. While similar co-management parameters between treatments (ISV vs. CONV) were tightly controlled for
560 within each site, some variation in co-management occurred between sites. Site 1 had the most variation from other
561 sites, with the lowest %clay and highest soil disturbance. Whereas most sites were managed without tillage, Site 1
562 received conservation tillage (i.e. shallow tillage of every other row in alternating years) and showed a much lower
563 distribution of >2000 um macroaggregates relative to other sites (Supplemental Figure 1). Further, while all sites
564 received planted cover crops, the seeding mixture composition slightly differed between years and sites. There is a
565 possibility that this co-management variation resulted in distinct and divergent outcomes between sites. However,
566 treatment(*x*)location interactions indicate notable similarities across sites, with soil exo-enzymes as the only
567 parameters showing significant treatment response variation between locations (Figure 3). These research
568 considerations are well understood and persistent challenges for developing ICL research platforms, especially given
569 the situational variability in on-the-ground cropland grazing applications (Tanaka et al., 2008).
570
571 Given this context, our study sought to evaluate the soil carbon flux dynamics and storage potential of long-term
572 perennial integrated crop-livestock management on working farms. We were particularly interested if, and to what
573 degree, ICL in perennial systems affects the partitioning of SOC into distinct biochemical and physical pools.
574 Further, we sought to understand whether long-term perennial ICL legacy effects impact microbial ecological
575 characteristics such as community structure, soil carbon utilization, and investment strategies related to biomass
576 accumulation, stress tolerance, and the production of soil exo-enzymes. We provide here supporting evidence that
577 small ruminant grazing can increase stable carbon storage within perennial cropland soils, especially when
578 accounting for the subsoil. Our findings also show that the continuous year-after-year use of perennial cropland
579 grazing altered soil carbon quality across four distinct vineyards, with a higher total quantity and greater relative
580 proportion of soil carbon allocated toward biologically active carbon flux pools – the carbon most readily available
581 and utilized by soil microbial communities. We argue that this stimulation in soil carbon flux is likely driven by
582 increased deposition of labile, soluble compounds (i.e. animal excreta and rhizodeposition) resulting from
583 successive years of high intensity rotational grazing events. This also presents a viable explanation as the dominant

584 driver of potential SOC accumulation in perennial ICL systems, due to enhanced production of microbial biomass,
585 accumulation of microbial necromass, and, therefore, increased stabilization as MAOC. These results indicate strong
586 potential of perennial ICL management to stimulate carbon (energy) flows and invigorate internal agroecosystem
587 processes, with significant relevance for climate change mitigation and adaptation goals in Mediterranean perennial
588 croplands.
589
590 Simultaneously,
591
592 Observing strong trends across sites and contexts is noteworthy, especially when considering the additional
593 variability tha
594
595 ***4.1 Perennial cropland grazing increased the active flux of soil carbon throughout the soil profile***
596 Our study shows that the introduction of sheep grazing increased the pool of actively fluxed soil carbon across four
597 distinct paired vineyards, with most significant impacts observed in subsoils. This was the case both in terms of the
598 total size of labile SOC pools and the relative proportion (% of total SOC) allocated toward labile carbon flux pools,
599 especially within the microbial biomass carbon (MBC) pool. This corroborates findings from other studies across
600 ICL systems, where positive impacts of cropland grazing on the size of soil microbial communities are commonly
601 reported (Acosta-Martínez et al., 2004; Bansal et al., 2022; da Silva et al., 2015; Franzluebbers and Stuedemann,
602 2008; Sekaran et al., 2021a; Silva et al., 2022; Tracy and Zhang, 2008). The benefits of sheep grazing for soil
603 microbial growth were observed at all depths, with an average MBC increase of 82%, 65%, and 99% at the 0–15,
604 15–30, and 30–45 cm depths, respectively (Figure 4B-C). While the MBC pool comprised a notably higher relative
605 proportion of total SOC at the surface soil (0–15 cm) under ISV (+49% *Qmic*; Figure 5A), the MBC pool was
606 otherwise most significantly impacted by ISV management in the subsoil.
607
608 Increases in MBC may be attributed to shifts in the grazed plant community's composition, productivity, and the
609 allocation of energy and nutrients above- and belowground (Bardgett and Wardle, 2003; Cong et al., 2014; Dawson
610 et al., 2009; Rumpel et al., 2015; Tian et al., 2016). While cropland-specific impacts are less understood, research

throughout diverse grazed ecosystems show that feedbacks between grazing *intensity* (density and duration) and *periodicity* (seasonality and frequency) exert unique selective pressure on plant communities and the rate and quality of carbon influxes (Figure 1). Numerous studies have documented higher rates of rhizodeposition immediately following high-intensity grazing events (Dawson et al., 2009; Gavrichkova et al., 2008; Hamilton et al., 2008; Hamilton and Frank, 2001), which increases the availability of labile and soluble carbon substrates and facilitates preferential and efficient utilization by soil microbial communities (Cheng and Kuzyakov, 2015; Gavrichkova et al., 2008; Ota et al., 2013; Wilson et al., 2018). Observed increases in MBC under ISV management may also be facilitated by the mineralization of aboveground plant residues within the ruminant of grazing animals – where significant quantities of recalcitrant plant structural compounds such as cellulose and lignin are fragmented, depolymerized, and returned to the soil as more labile, soluble, and nutrient-dense excreta (dung and urine) (Faissal et al., 2017; Jarvis, 2009; Soussana and Lemaire, 2014). Ruminant mineralization and trampling of plant residues also likely explain the lower quantities of POC found in the surface soils of the ISV treatment (Figure 7G).

The effects of ISV management on the availability and active microbial utilization of labile soil carbon were generally most pronounced in the subsoil, which may result from increased leaching of soluble DOC compounds deeper into the soil profile. This assumption is supported by our observations of increased trends in dissolved organic carbon (DOC) content (Figure 4F) and potentially mineralizable carbon (PMC; Figure 4H-I) in subsurface soil depths under ISV relative to CONV understory management, as well as other studies reporting higher DOC concentrations under various ICL management systems (Sekaran et al., 2021a; Tian et al., 2010). Due to their enhanced transport within soil solution, compounds in the DOC pool are generally more spatially accessible to microbial processing (Erktan et al., 2020; Nakhavali et al., 2021; Neff and Asner, 2001; Ota et al., 2013; Rumpel and Kögel-Knabner, 2011). The lower molecular weight and activation energy requirements of compounds in the DOC pool also facilitate quick microbial assimilation and utilization than the complex and less nutrient-rich structural compounds associated with the POC pool (Blagodatskaya et al., 2011; Kallenbach et al., 2016, 2015; Kok et al., 2022; Lavallee et al., 2020; Shahbaz et al., 2017; Weiss et al., 1991). Soil carbon mineralization rates did not vary between treatments at any depth relative to the SOC resource pool size of each vineyard ($Cmin_{soc}$; Figure 6D-F), indicating similarities in overall SOC quality regardless of differences in SOC pool size. Higher total soil carbon

638 flux rates in the 15–30 cm subsoil depth under ISV management (Figure 6B), measured via increased cumulative
639 rates of soil carbon mineralization ($Cmin_{total}$), is likely related to overall larger labile soil carbon pool sizes under
640 grazed ISV compared to CONV vineyards. The larger MBC pool within the ISV treatment may also more
641 positioned to rapidly utilize labile and physically accessible soil carbon pools under ideal soil moisture and
642 temperature conditions relative to the CONV managed soils (Geyer et al., 2020). This may partially explain the
643 higher PMC and rates of $Cmin_{total}$, despite a lack of observed differences in DOC content between treatments at both
644 0–15 and 15–30 cm soil depths.
645
646 Whereas nutrients from excreta may be more readily transformed and assimilated by soil microbes than ungrazed
647 plant residues (Kooch et al., 2020; Wang et al., 2018), ruminant mineralization of plant residues has also been
648 shown to increase the bioavailability of soil N and P for plant and microbial uptake as a result of high-intensity
649 grazing disturbance events (Costa et al., 2014; Tracy and Frank, 1998; Wu et al., 2011; Zhang et al., 2020). We
650 observed substantially higher quantities of extractable P at surface soil depths and soluble mineral N content (NH_4
651 and NO_3) in subsoils at all ISV sites (Table 2). The depth stratification of significant treatment differences between
652 these nutrient pools likely reflect differences in solubility of N and P and their physical transport pathways within
653 soil solution. Future research should explore the impacts of perennial ICL management on net primary productivity
654 (NPP) of the understory plant community. Positive feedback mechanisms between soil N and P bioavailability and
655 enhanced plant productivity are well established. However, it is unclear whether, and the degree to which, ISV
656 grazing ecophysiology and alterations in nutrient bioavailability (via animal-derived manure and urine deposition)
657 stimulate understory NPP and increase plant-derived carbon inputs relative to the ungrazed vineyards. In either case,
658 the increased bioavailability of soil N and P, coupled with intra-ruminal conversion of POC (*celluloses, hemi-*
659 *celluloses, lignin, etc.*) to DOC and altered C influx pulses from rhizodeposition, are all potential mechanisms
660 underlying observed increases in soil microbial biomass and their rates of carbon mineralization under ISV
661 management.
662
663 ***4.2 Grazing shifted the resource investment strategy of surface soil microbial communities toward efficient***
664 ***biomass accumulation***

Our results indicate that perennial cropland grazing stimulated microbial growth efficiency and biomass accumulation. A general framework to interpret this observation is with consideration of how soil habitat and resource conditions orient microbial energy investment strategies (Ho et al., 2017). As soil ecosystem conditions shift in response to agroecosystem management disturbances, this influences microbial metabolic processes related to tolerance of stressors, the acquisition of resources, and capacity for growth. When less energy is required to maintain functionality under limited resources and inhospitable conditions, soil microbial communities are more likely to allocate energy toward biomass accumulation (Malik et al., 2020). Increased microbial biomass carbon (MBC) in surface soils under ISV was paired with remarkably higher total quantities of PLFA biomarkers – a 47% higher value relative to the CONV treatment (Table 3). We further observed 41% lower average metabolic quotient (Q_{met}) values (Figure 5C) in surface soils of grazed vineyards, which indicates higher microbial carbon use-efficiency (CUE) as less CO_2-C is respired relative to the size of the MBC pool (Dilly and Munch, 1998; Sinsabaugh et al., 2017). Increased availability of soil N and P relative to C have been shown to lower Q_{met} values across climatic and soil management gradients (Xu et al., 2017). Increased microbial growth efficiency and investment in biomass accumulation under ISV management is further supported by the substantially higher proportion of MBC relative to total SOC (+49% *Qmic*) within the 0–15 cm depth (Figure 5A). Higher Q_{mic} values are associated with a greater potential for soil microbes to transform energy sources via increased availability of soil carbon and nutrients (Sparling, 1992; Sun et al., 2020). Trends toward higher PMC values are also indicative of increased energy source availability in the grazed vineyards, as this pool measures the reservoir of readily available soil carbon that drives microbial functions (catabolism) and biomass accumulation (anabolism) (Levi-Minzi et al., 1990). These findings corroborate another recent ICL study, which showed higher microbial biomass in grazed cropland that was similarly attributed to increased availability of carbon and nutrient substrates ((Sekaran et al., 2021a)). Given the role of microbial necromass in the formation of stable MAOC, these efficiency and growth indicators suggest a higher net SOC storage potential under ISV management.

As another indicator of microbial investment, the production of metabolically-costly extra-cellular enzymes represent shifts in resource acquisition strategies in response to growth factor limitations through altering energy and nutrient availability within the near-cell soil environment (Nannipieri et al., 2002; Zheng et al., 2020). While the

availability of soil P was higher in surface soils of the ISV treatment (Table 2), we observed significantly lower enzymatic activity related to P cycling (phosphatase; Figure 3C). The composition of sheep excreta has high inorganic P content (Arnuti et al., 2020) and soil applications of inorganic P have been shown to reduce phosphatase activity (Oshima et al., 1996), as this enzyme cleaves phosphate (PO_4) groups from proteins and becomes an increasingly unnecessary investment under high P availability conditions. These observations indicate an increased accessibility of soil P and reduced microbial investment in P acquisition (Nannipieri et al., 2011) in perennial croplands with grazing. At the same time, we observed significantly higher N cycling enzymatic activity in the ISV treatment as measured by aminopeptidase (peptide hydrolysis) and urease (urea hydrolysis) enzymes (Figure 3A-B). The increase in urease activity corroborates previous findings across various grazed ecosystems (Acosta-Martínez et al., 2010; McNaughton et al., 1997; Sekaran et al., 2021a) as a result of urea degradation, the dominant N constituent found in urine (Bristow et al., 1992). The release of aminopeptidase enzymes could indicate potential limitation in microbial N availability and an increased metabolic investment in N acquisition (Schimel and Bennett, 2004). However, both the mineral N (NO_3 and NH_4) pool and the higher total N content of ISV surface soils (0–15 cm) do not indicate reduced availability of soil N (Table 2). Alternatively, recent research has suggested that soil microbial communities also use aminopeptidase enzymes as a means to access protein-derived carbon – a potent energetic resource for cellular growth (Norman et al., 2020).

Shifts in microbial processes may reflect differences in the abundance of core functional groups such as those related to decomposition (Bhatti et al., 2017; Setälä and McLean, 2004), which were higher in the ISV treatment across both fungal (+53% saprophytic fungi) and bacterial (+64% actinomycete) groups (Table 3). While neither fungal/bacterial ratios nor the mole percent distribution (mol%) of fungal PLFAs were significantly different between treatments, we did observe a significant increase in mol% of bacterial PLFAs. Traditional soil food web models assume distinct and preferential utilization of recalcitrant (slow energy channel) and labile (fast energy channel) carbon substrates by fungal and bacterial groups, respectively (Hunt et al., 1987). Under this view, changes in the quantity and quality of organic inputs should therefore induce shifts in soil fungal/bacterial ratios (Wardle et al., 2004). Alternatively, emerging empirical evidence has shown that multi-channel omnivores are the dominant constituency of both fungal and bacterial communities and the presence of these omnivores help to stabilize soil

food web communities (Kramer et al., 2016; Wolkovich, 2016). Conceptual models also indicate that fungal and bacterial communities can coexist in a stable state with the presence of large labile carbon pulse inputs, such as the input of dung and urine over the course of a grazing event (de Vries and Caruso, 2016), though bacteria still likely hold a competitive advantage in utilizing these substrates (Ho et al., 2017; Xun et al., 2018). While we observed benefit for both bacteria and fungi with vineyard grazing, relative increases in bacterial groups under ISV nevertheless suggest a soil ecosystem (habitat and/or resource) shift that preferentially benefit consumers of labile substrates and fast energy channels.

Within bacterial communities, the Gram(+)/Gram(-) ratio is thought a useful indicator of environmental disturbance along the r-K-strategist spectrum. Gram(+) bacteria are generally more adapted to heavily disturbed soil environments (habitat and/or resource limitation) and often less dependent than Gram(-) bacteria on the continuous input of labile carbon compounds (De Vries and Shade, 2013; Fanin et al., 2019). We observed no significant variation in the Gram(+)/Gram(-) ratio between treatments which, when analyzed in tandem with fungal/bacterial ratios, suggests no variation in the stress response of soil microbial communities as a result of cropland grazing. This is further supported by other measured soil physicochemical indicators such soil water content, pH, and compaction (bulk density; BD) – which were similar amongst treatments and indicate physical habitat conditions that are relatively alike (Table 2).

.3 Integrated crop-livestock grazing increased perennial cropland soil carbon storage in subsoils

Total SOC storage trended higher in the ISV treatment at both subsurface depth zones. The treatment effect was more significant with increasing depth and resulted in a 39% and 34% increase in SOC under ISV compared to the CONV treatment at 15–30 and 30–45 cm, respectively (Figure 7B-C). The introduction of sheep grazing into perennial cropland increased the physicochemical stabilization of soil carbon within the mineral matrix (MAOC) of the deepest measured subsoil layer (30–45 cm) by 37% compared to the CONV treatment (Figure 7F). The larger soil carbon storage response in the subsoil from grazing reflect changes in soil carbon flux pools (Figure 9B) and are likely related to the increased solubility of deposited animal excreta and deeper spatial distribution of DOC and nutrient substrates (Gross and Harrison, 2019; Rumpel and Kögel-Knabner, 2011). The higher CUE of grazed soils,

as indicated by lower Q_{met} values under ISV in the 0–15 and 30–45 cm depths (Figure 5E-F), should theoretically build the capacity for SOC storage (Figure 1B). Where both the rate and efficiency of microbial carbon utilization is higher for labile urine and manure inputs than plant structural compounds (POC) (Cai et al., 2016; Hossain et al., 2017), these properties have been shown to facilitate a more direct pathway toward long-term MAOC stabilization and persistence (Cotrufo et al., 2015, 2013; Dynarski et al., 2020; Haddix et al., 2016; Lavallee et al., 2020; Liebmann et al., 2020). The incorporation of litter-derived POC by animal trampling has also recently been shown to increase its microbial utilization, expediting its decomposition rate and promoting increased physiochemical stabilization of MAOC (Wei et al., 2021).

However, the introduction of ruminant grazers did not result in significant alterations to surface soil (0–15 cm) aggregation (Figure 3), despite both the physical disturbance of animal trampling and grazing-induced reductions in POC content (Figure 7G). This is notable, given that POC is essential as a nucleus in the formation and stability of soil macroaggregates (Six et al., 2000). We also did not show significant differences in surface soil (0–15 cm) aggregate-associated C between ISV and CONV treatments, as represented by both the total C content (g kg^{-1}) associated with soil aggregate size fractions (Figure 8A-D) and the relative distribution (% of total C) across those size fractions (Supplementary Figure 2). As a measurement of the SOC pool associated with four distinct aggregate size categories, aggregate-associated C is an indicator of SOC physical protection via occlusion within aggregates. As such, this study does not indicate that aggregate occlusion of SOC is strongly impacted by perennial ICL grazing.

Our results corroborate some previous ICL research findings, where increases in SOC have been reported under crop-livestock integration across a spectrum of crop production systems (Acosta-Martínez et al., 2010, 2004; Bansal et al., 2022; Da Silva et al., 2014; de Faccio Carvalho et al., 2010; Franzluebbers et al., 2014; Fultz et al., 2013; Maughan et al., 2009). Given the variability in ICL grazing intensity (density and duration) and its interactions with climatic, edaphic, and co-management components across agricultural systems, other studies across diverse ICL systems have also found negligible (Fernández et al., 2011; Liebig et al., 2020; Tian et al., 2010) and even negative (Tobin et al., 2020) SOC storage benefits associated with cropland grazing. As empirical evidence increasingly shows the positive relationship between soil microbial growth and SOC formation and stabilization (Bradford et al.,

2013; Kallenbach et al., 2016, 2015; Wang et al., 2021), agroecosystem design and management characteristics that stimulate microbial biomass formation and necromass preservation may be central toward increasing SOC storage (Lange et al., 2015; Liang et al., 2019; Prommer et al., 2020; Wilson et al., 2018). When metabolic investment trade-offs (i.e. less energy to invest elsewhere) are satisfied through ameliorating soil habitat and nutrient limitations, this may facilitate efficient microbial biomass accumulation strategies (Malik et al., 2020). The increased allocation of soil carbon toward active microbial pools, with higher use-efficiency, may indicate a determinant mechanism necessary for increasing SOC accumulation within grazed perennial cropland (Figure (9).

5. *CONCLUSION*

Minimal attention has been paid toward perennial ICL systems and even less so toward the differing dynamics of the surface soils and subsoils within these systems. As such, this research provides some of the first insights into the potential SOC storage benefits associated with perennial cropland grazing, particularly within subsoils. Our study results provide early evidence that *high-density, short-duration rotational grazing* management in perennial croplands holds significant potential to increase SOC storage. We propose increased rates and efficiency of microbial carbon accrual as underlying mechanisms to explain observed gains in SOC. However, these outcomes differ across soil depths and are strongly influenced by the intensity and periodicity (seasonality and frequency) of grazing events as well as site-specific edaphic and climatic limitations. Soil biogeochemical outcomes may therefore be highly variable under different agroecosystem and grazing management regimes and how they are synchronized across time and space. Considerations of co-management strategies such as understory species composition and tillage regime will partially determine soil habitat and resource conditions. The strategic application of grazing in coordination with knowledgeable shepherding practitioners is likely necessary to optimize potential soil benefits. Where perennial cropland agroecosystem design and management may be easily altered to facilitate both spatial and temporal livestock re-integration, better understanding of the mechanistic pathways between grazing disturbances and cropland SOC cycling will be useful toward strategically improving the internal regulation of soil functions and increasing longer-term SOC storage. As such, we aim to highlight certain benefits of using updated soil carbon conceptual frameworks for linking SOC flux and storage indicators within applied agricultural research contexts. However, measurements of soil carbon flux are extremely dynamic across time and space. Future research should

focus on monitoring trials and capturing multiple sampling points over shorter durations, to better elicit specific cropland soil responses to grazing disturbance events.

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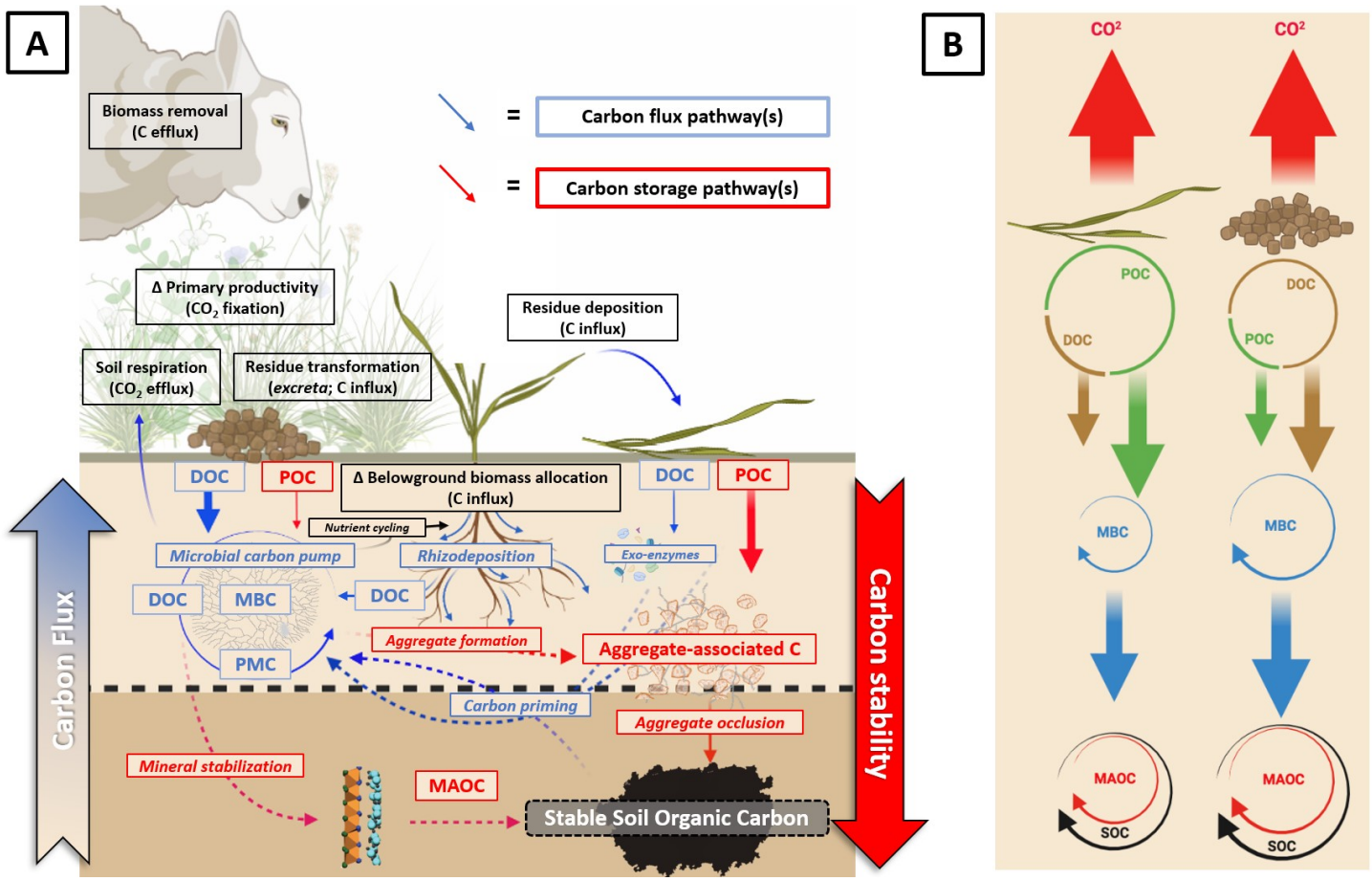
TABLES AND FIGURES

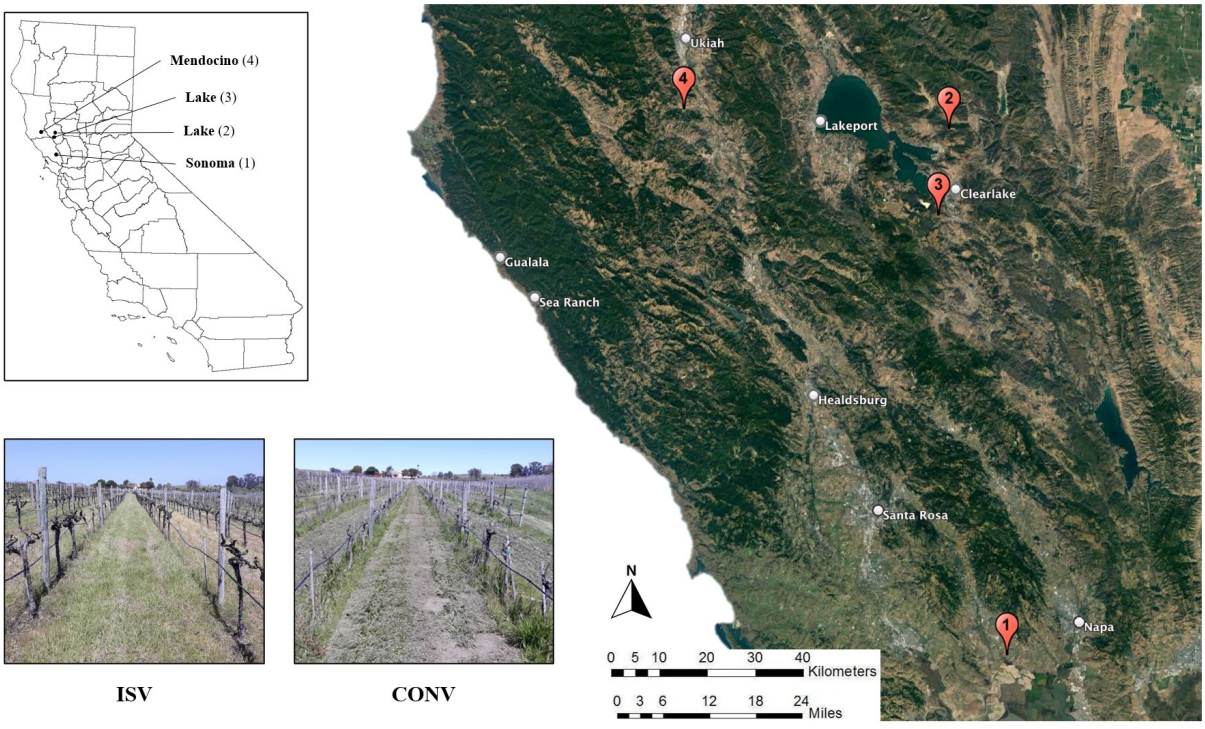
Figure 1: Conceptual framework for soil carbon flux and storage pathways in perennial integrated crop-livestock systems



A. Potential direct mechanisms by which sheep grazing on understory forage alters carbon (C) flows into perennial cropland soils (black). Once deposited (C influx), labile and physically accessible soil organic carbon (SOC) is fluxed (blue) through the soil ecosystem via the microbial carbon pump (MCP). Within the MCP, soil C is continually transformed and (re)cycled through assimilation within microbial biomass carbon (MBC), the release of dissolved organic carbon (DOC) substances and/or formation of microbial necromass, and re-assimilation back into MBC. This potentially mineralizable carbon (PMC) may continually cycle within the MCP or "leak" from the pump through either respiration (CO_2 efflux) or various soil carbon storage pathways (red). SOC storage pathways include mineral stabilization via formation of mineral-associated organic carbon (MAOC) or aggregate occlusion and physical protection as aggregate-associated C. Particulate organic carbon (POC) may also enter the MCP through fragmentation and depolymerization or may take a more direct storage pathway via aggregate occlusion. Storage pools may also re-mobilize into flux pools via soil carbon priming. **B.** Compared to the original plant residues, animal excreta from ruminant grazing of forage represents a smaller portion of the original photosynthetically fixed

C content. Though lower in total C content compared to plant residues, the carbon quality of animal excreta is characterized by a higher proportion of soluble, nutrient-rich, and labile dissolved organic carbon (DOC) substances. These DOC substances are more reactive, easily diffusible throughout the soil profile, and readily available for assimilation as microbial biomass carbon (MBC). The higher microbially carbon use-efficiency of DOC compared to particulate organic carbon (POC) substances may potentially facilitate a more direct pathway toward stabilized mineral-associated organic carbon (MAOC) storage, and therefore higher total soil organic carbon (SOC) storage.

Figure 2: Map of study region



Paired vineyard sites (4 locations) consisted of one '*non-integrated*' vineyard (understory vegetation managed through mowing and herbicides; CONV) and one adjacent '*integrated*' vineyard (understory vegetation managed through grazing; ISV), with one location in Sonoma County (1), two in Lake County (2 & 3), and one in southern Mendocino County (4), California, USA. Photos show vineyard comparisons immediately after occurrence of grazing (ISV) and mowing (CONV) events. Aerial imagery of the study area was derived from Google Earth Pro.

Table 1: ISV survey site characteristics of 8 paired vineyards in Sonoma, Lake, and Mendocino Counties, California, USA

Site	Understory management treatment	Length of current management (years)	Vine [varietal/ rootstock] (vineyard plant date)	Soil texture[a] (% clay)	Soil type[b] (% slope)	Soil disturbance[c]	Synthetic herbicide application[d]	CCOF[e] organic certification status
1	ISV	17	Pinot Noir [UCD 12 / 1103P] (2001)	Loam (22%)	Haire (0-9%)	High	No	Yes
	CONV	21	Pinot Noir [UCD 13 / 1103P] (1997)	Loam (21%)	Haire (0-9%)	High	Yes	No
2	ISV	14	Cabernet Sauvignon [337 / 1103] (2001)	Clay Loam (28%)	Benridge -Sodabay (15-30%)	Moderate	No	No
	CONV	8	Cabernet Sauvignon [15 / 1103] (1998)	Clay Loam (28%)	Benridge -Sodabay (15-30%)	Low	Yes	No
3	ISV	14	Cabernet Sauvignon [08 / 110R] (1992)	Clay Loam (33%)	Sobrante -Guenoc-Hambright (15-30%)	Moderate	No	No
	CONV	21	Cabernet Sauvignon [CS7 /110R] (1996)	Clay Loam (29%)	Sobrante -Guenoc-Hambright (15-30%)	Low	Yes	No
4	ISV	17	Chardonnay [76 / 5C] (2001)	Loam (25%)	Cole (2-5%)	Low	No	Yes
	CONV	10	Chardonnay [76 / 5C] (2003)	Loam (27%)	Cole (2-5%)	Low	Yes	No

[a] Based from hydrometer measured sand, silt, and clay particle content (0–15 cm)

[b] Haire clay loam (0 to 9% slope): clayey, mixed, thermic Typic Haploxerults; Benridge-Sodabay loams (15 to 30% slope): (Benridge) fine, mixed, thermic Mollic Palexeralfs / (Sodabay) fine-loamy, mixed, thermic Mollic Haploxeralfs; Sobrante-Guenoc-Hambright complex (15 to 30% slope): (Sobrante) fine-loamy, mixed, thermic Mollic Haploxeralfs / (Gueonic) fine, kaolinitic, thermic Typic Rhodoxeralfs / (Hambright) loamy-skeletal, mixed, thermic Lithic Haploxerolls. Collected from the USDA-NRCS *SoilWeb* app (https://casoilresource.lawr.ucdavis.edu/)

[c] Soil disturbance is categorized as *Low* (infrequent mow or graze), *Moderate* (combination of infrequent mow + graze), and *High* (combination of infrequent mow or graze + conservation tillage [i.e. shallow tillage of every other row in alternating years])

[d] Applications occurring in vineyard undervine row only. Vineyard interrow was managed exclusively with grazing or mowing

[e] California Certified Organic Farmers (CCOF) – United States Department of Agriculture certifying agency

[*] *All vineyards contained planted cover crops in vineyard interrow, but not in undervine row*

[*] *No vineyards contained either organic or synthetic fertilizer amendments within vineyard interrow*

Table 2: Physicochemical properties from integrated sheep-vineyard (ISV) and conventional understory (CONV) managed soils

Depth (cm)	Location (Site #)									
	Sonoma (1)		Lake (2)		Lake (3)		Mendocino (4)			
	ISV	CONV	ISV	CONV	ISV	CONV	ISV	CONV	Treatment	Treatment x Location
Total N (g kg^{-1}) [Total C:N]									(*p*-value)	(*p*-value)
0–15	2.0* [10.4]	1.3 [10.6]	1.8 [14.1]	1.6 [15.8]	2.3* [13.5]	1.6 [16.5]	2.2 [12.5]	1.8 [12.5]	0.013* [0.649]	0.620 [0.954]
15–30	1.2* [9.9]	0.8 [8.3]	0.7 [13.6]	0.8 [13.3]	0.9 [14.6*]	0.6 [12.1]	1.3 [11.7]	0.9 [12.4]	0.228 [0.593]	0.885 [0.750]
30–45	0.9 [8.8]	0.7 [7.1]	0.5 [12.6***]	0.5 [8.8]	0.5 [12.6***]	0.5 [9.4]	1.1 [12.3]	0.9 [12.2]	0.694 [0.084]	0.939 [0.430]
Mineral N ($NH_4^+ + NO_3^-$) (mg kg^{-1})										
0–15	16.6	15.1	13.5***	5.9	21.2***	8.4	5.2	5.0	0.183	0.975
15–30	9.2*	5.2	4.2	4.0	7.7***	2.7	4.4	4.1	0.211	0.672
30–45	7.6**	4.7	2.4	1.4	5.2*	2.7	5.0	4.3	0.042*	0.750
Extractable P (ug g^{-1})										
0–15	26.4* (3.5)	21.2 (3.4)	11.6* (2.6)	6.8 (0.8)	19.24* (1.2)	10.8 (1.6)	16.2** (1.3)	7.2 (0.9)	<0.001 ***	0.443
15–30	9.8 (3.5)	9.3 (2.7)	4.8 (0.3)	4.3 (1.0)	11.0*** (1.3)	3.8 (0.7)	8.0 (0.9)	8.0 (0.9)	0.309	0.985
30–45	7.1 (2.0)	6.1 (2.0)	4.8 (0.4)	4.6 (0.8)	9.1** (1.3)	3.5 (0.2)	6.5 (0.5)	10.5 (2.7)	0.371	0.789
Bulk density (g cm^{-3})										
0–15	1.54 (0.04)	1.64 (0.02)	1.28 (0.04)	1.25 (0.03)	1.20 (0.01)	1.30 (0.02)	1.28 (0.04)	1.27 (0.04)	0.634	0.966
Soil water content (g g^{-1})										
0–15	0.25 (0.01)	0.19 (0.01)	0.21 (0.004)	0.16 (0.01)	0.30 (0.01)	0.27 (0.01)	0.08 (0.01)	0.08 (0.004)	0.798	0.840
15–30	0.20 (0.01)	0.18 (0.01)	0.24 (0.004)	0.20 (0.02)	0.30 (0.01)	0.28 (0.01)	0.11 (0.01)	0.09 (0.01)	0.777	0.844
30–45	0.22 (0.01)	0.22 (0.02)	0.27 (0.02)	0.27 (0.02)	0.27 (0.01)	0.31 (0.01)	0.12 (0.01)	0.11 (0.01)	0.851	0.931
pH										
0–15	7.28 (0.04)	7.49 (0.09)	6.79 (0.08)	6.88 (0.08)	6.43 (0.10)	6.51 (0.17)	6.69 (0.10)	6.60 (0.10)	0.738	0.655
15–30	7.00 (0.10)	6.98 (0.12)	6.96 (0.10)	7.01 (0.08)	6.61 (0.17)	6.69 (0.09)	6.48 (0.04)	6.81 (0.08)	0.678	0.254
30–45	7.01 (0.07	6.93 (0.07)	7.00 (0.15)	7.14 (0.05	6.80 (0.22)	6.83 (0.08)	6.60 (0.03)	6.51 (0.13)	0.416	0.325
EC (dS m^{-1})										
0–15	0.25*** (0.03)	0.14 (0.01)	0.16* (0.005)	0.11 (0.01)	0.22*** (0.03)	0.11 (0.01)	0.12 (0.02)	0.10 (0.005)	0.058	0.153
15–30	0.21*** (0.02)	0.09 (0.01)	0.12* (0.02)	0.08 (0.01)	0.08 (0.01)	0.10 (0.02)	0.07 (0.01)	0.06 (0.004)	0.121	0.043*
30–45	0.27*** (0.03)	0.14 (0.03)	0.11 (0.01)	0.09 (0.01)	0.09 (0.01)	0.12 (0.02)	0.06 (0.004)	0.06 (0.003)	0.353	0.138
CEC (me 100g^{-1})										
0–15	16.6*** (1.8)	11.2 (0.6)	14.8 (0.5)	14.3 (0.4)	15.8 (0.8)	18.1 (0.8)	18.2 (0.7)	17.8 (0.4)	0.270	0.041*
15–30	18.3*** (1.4)	11.9 (0.9)	13.7 (0.9)	13.2 (0.5)	13.8 (0.9)	15.1 (0.9)	15.3 (1.2)	19.6 (0.7)	0.333	0.203
30–45	22.0*** (1.7)	15.5 (2.6)	13.5 (0.8)	14.1 (0.9)	13.3 (0.8)	16.3 (0.3)	19.5 (1.7)	19.4 (0.07)	0.637	0.278

Soils were sampled across 8 paired vineyards (4 locations). Soils cores were separated into three depths zones (0–15 cm, 15–30 cm, and 30–45 cm) and measured for total N (TN), mineral N (NH_4^+-N plus NO_3^-N), extractable phosphorous (P), bulk density, soil water content, pH, electrical conductivity (EC), and cation exchange capacity (CEC). Means are followed by standard error in parentheses. Shown are the treatment and treatment(x)location statistical significance across sites (n=64). For each location, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Asterisks (*) denote significant treatment differences at each depth increment.

* p <0.05; ** p <0.01; *** p < 0.001.

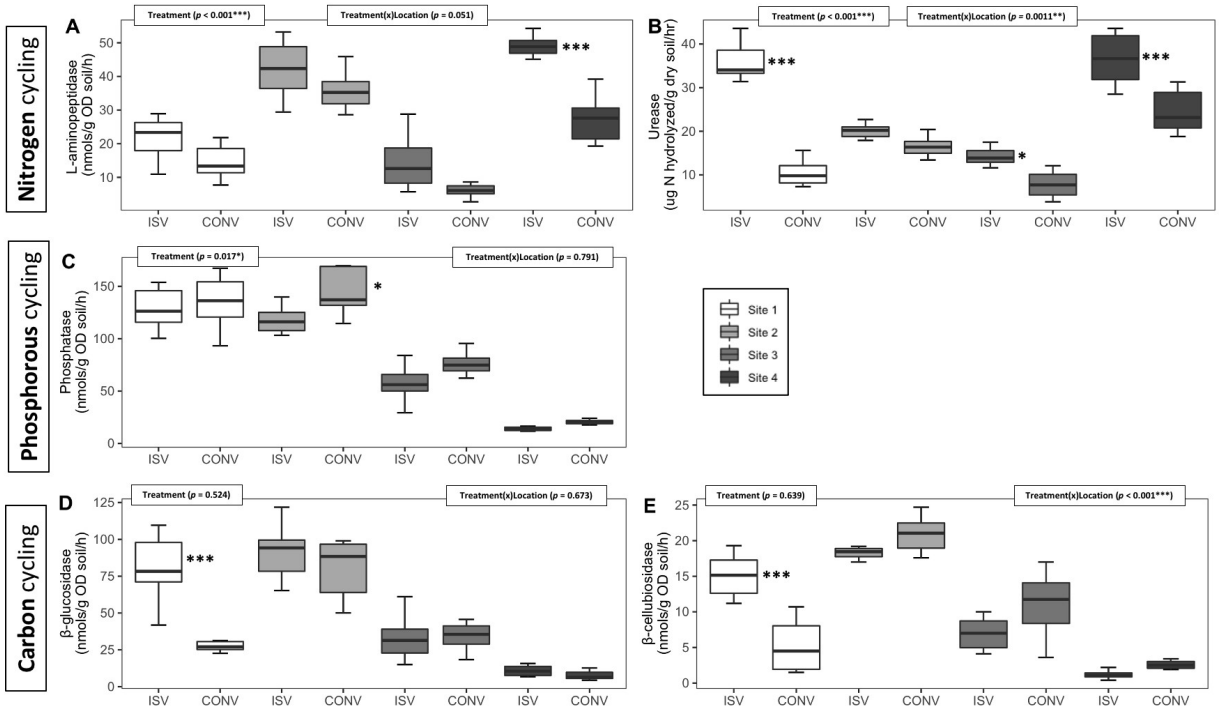
Table 3: PLFA biomarkers and biological ratios from integrated sheep-vineyard (ISV) and conventional understory (CONV) surface soils (0–15 cm depth)

	Location (Site #)									
	Sonoma (1)		Lake (2)		Lake (3)		Mendocino (4)			Treatment x Location
	ISV	CONV	ISV	CONV	ISV	CONV	ISV	CONV	Treatment	
Total PLFAs									(*p*-value)	(*p*-value)
ng g^{-1}	3628* (632)	2560 (252)	5408* (227)	4341 (206)	3594* (470)	2338 (396)	1540 (535)	934 (263)	<0.001 ***	0.613
Fungi										
ng g^{-1}	457 (119)	252 (45)	625 (23)	454 (55)	451 (88)	332 (54)	7 (6)	12 (9)	0.004**	0.062
mol%	11.8 (1.7)	9.3 (1.4)	11.7 (0.5)	10.4 (1.1)	12.5 (1.5)	15.8 (3.7)	0.6 (0.4)	0.8 (0.5)	0.991	0.749
AM fungi										
ng g^{-1}	153 (34)	66 (12)	192 (9)	145 (17)	143 (25)	146 (67)	8 (6)	7 (6)	0.544	0.519
mol%	4.0 (0.6)	2.4 (0.4)	3.6 (0.2)	3.3 (0.3)	3.9 (0.3)	3.8 (0.7))	0.7 (0.5)	0.5 (0.3)	0.647	0.382
Saprophytic fungi										
ng g^{-1}	304 (89)	187 (34)	433* (23)	309 (42)	308 (65)	186 (38)	0 (0)	0 (0)	0.004 **	0.197
mol%	7.9 (1.2)	6.9 (1.1)	8.1 (0.5)	7.1 (0.9)	8.6 (1.2)	7.9 (1.2)	0 (0)	0 (0)	0.276	0.576
Bacteria										
ng g^{-1}	1734* (318)	1095 (117)	2380* (124)	1737 (102)	1528* (212)	788 (142)	346 (107)	205 (51)	<0.001***	0.161
mol%	47.3 (1.1)	42.6 (0.7)	43.9 (0.8)	40.0 (1.3)	42.0* (1.4)	33.6 (3.3)	22.2 (2.6)	21.7 (2.8)	0.002**	0.484
Actinomycetes										
ng g^{-1}	271* (45)	151 (18)	350 (25)	281 (14)	217** (31)	83 (15)	91 (21)	50 (12)	<0.001***	0.270
mol%	7.5 (0.2)	6.0 (0.4)	6.5 (0.3)	6.5 (0.4)	6.0* (0.3)	3.6 (0.5)	6.4 (1.1)	5.4 (0.6)	0.223	0.781
Fungi:bacteria ratio										
	0.25 (0.03)	0.22 (0.03)	0.27 (0.01)	0.26 (0.03)	0.30 (0.03)	0.34 (0.03)	0.07 (0.05)	0.06 (0.03)	0.887	0.680
Gram(+):gram(–) bacteria ratio										
	1.22 (0.12)	1.51 (0.34)	1.05 (0.04)	1.07 (0.07)	1.22 (0.10)	1.06 (0.09)	2.48 (0.43)	1.82 (0.22)	0.384	0.021*
Saturated:unsaturated ratio										
	1.54 (0.17)	2.27*** (0.47)	1.47 (0.06)	1.69 (0.12)	1.74 (0.20)	1.62 (0.11)	10.96 (1.96)	9.84 (1.99)	0.909	0.319

Phospholipid fatty acid (PLFA) profiles indicative of fungi, arbuscular mycorrhizal (AM) fungi, saprophytic fungi, bacteria, and actinomycetes, as well as the relative ratios of fungi-to-bacteria and stress indicator ratios gram(+)-to-gram(-) bacteria and saturated-to-unsaturated fatty acids. Ratios are unitless, while PLFAs are given in both ng g soil^{-1} and mole percent distribution (mol%). Means are followed by standard error in parentheses. Shown are the treatment and treatment(x)location statistical significance across sites (n=64). For each location, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Asterisks (*) denote significant treatment differences at each depth increment.

* p <0.05; ** p <0.01; *** p < 0.001.

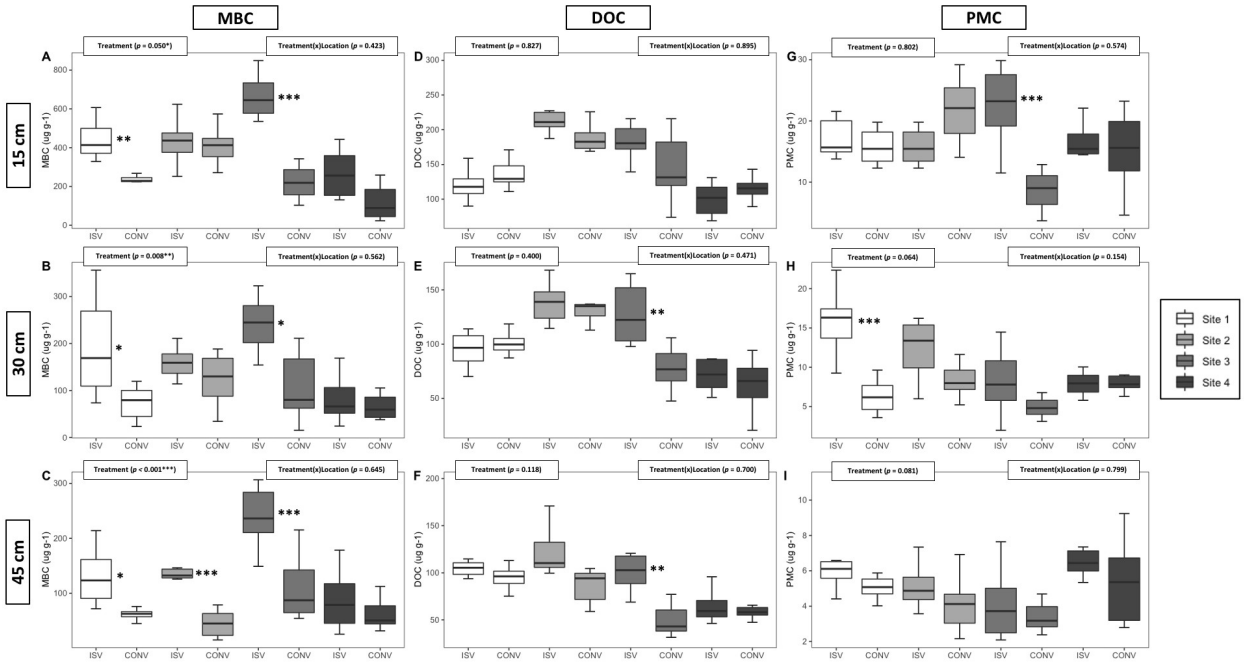
Figure 3: Management impact on soil exo-enzyme activity potential from surface soil (0–15 cm depth)



Impact of integrated sheep-vineyard (ISV) and conventional vineyard understory (CONV) management on surface soil (0 – 15 cm depth) exo-enzyme synthesis potential for (A) L-aminopeptidase, (B) urease, (C) phosphatase, (D) ß-glucosidase, and (E) ß-cellubiosidase. Shown are the treatment and treatment(x)location statistical significance across sites (n=64). For each location, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Error bars represent standard error. Asterisks (*) denote significant treatment differences at each depth increment.

* p <0.05; ** p <0.01; *** p < 0.001.

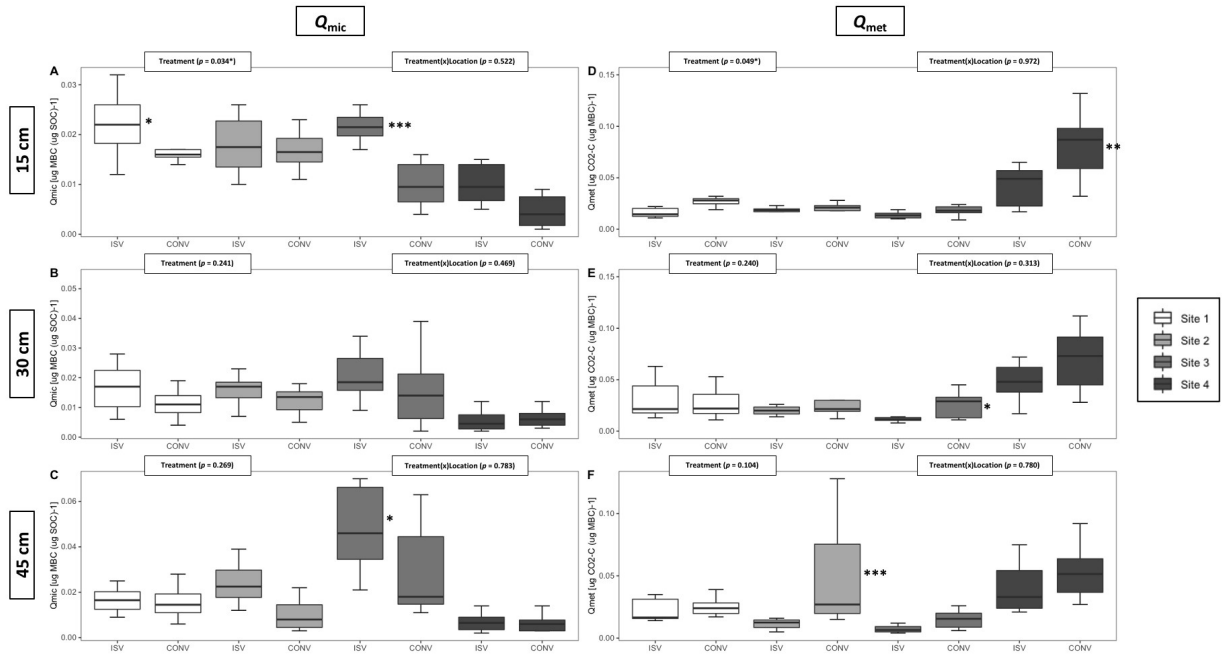
Figure 4: Soil carbon flux pools in integrated sheep-vineyard (ISV) and conventional understory (CONV) managed soils



(A-C) Microbial biomass C (MBC), (D-F) dissolved organic C (DOC), and (G-I) 3-day potentially mineralizable C (PMC) were measured at three depths zones (0–15cm, 15–30cm, and 30–45cm) from integrated sheep-vineyard (ISV) and conventional vineyard understory (CONV) managed soils. Shown are the treatment and treatment(x)location statistical significance across sites (n=64). For each location, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Error bars represent standard error. Asterisks (*) denote significant treatment differences at each depth increment.

* p <0.05; ** p <0.01; *** p < 0.001.

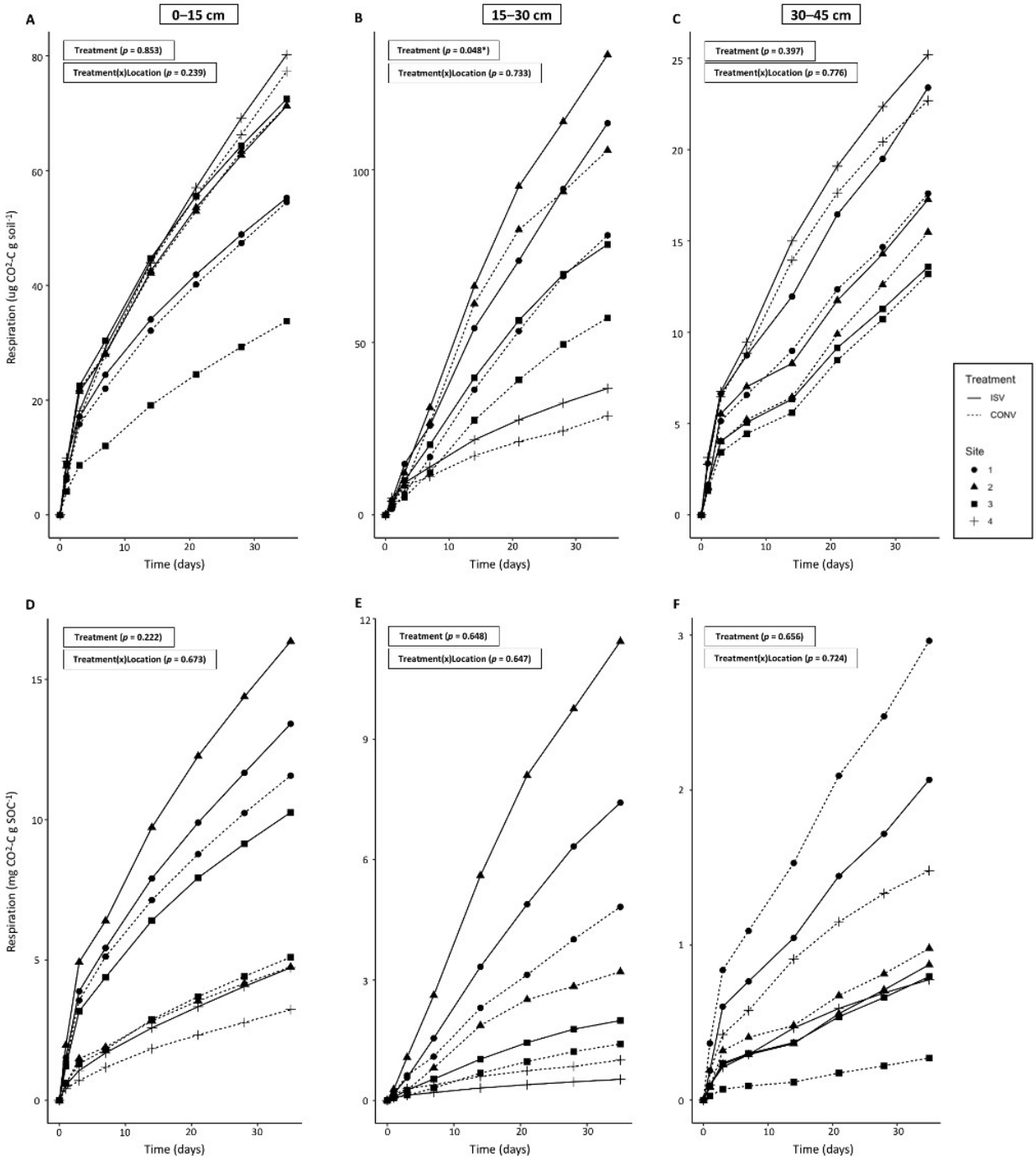
Figure 5: Microbial quotient (Q_{mic}) and metabolic quotient (Q_{met}) of integrated sheep-vineyard (ISV) and conventional understory (CONV) managed soils



(A-C) Microbial quotient (Q_{mic}) and (D-F) metabolic quotient (Q_{met}) were measured at three depths zones (0–15cm, 15–30cm, and 30–45cm) from integrated sheep-vineyard (ISV) and conventional vineyard understory (CONV) managed soils. Shown are the treatment and treatment(x)location statistical significance across sites (n=64). For each location, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Error bars represent standard error. Asterisks (*) denote significant treatment differences at each depth increment.

* p <0.05; ** p <0.01; *** p < 0.001.

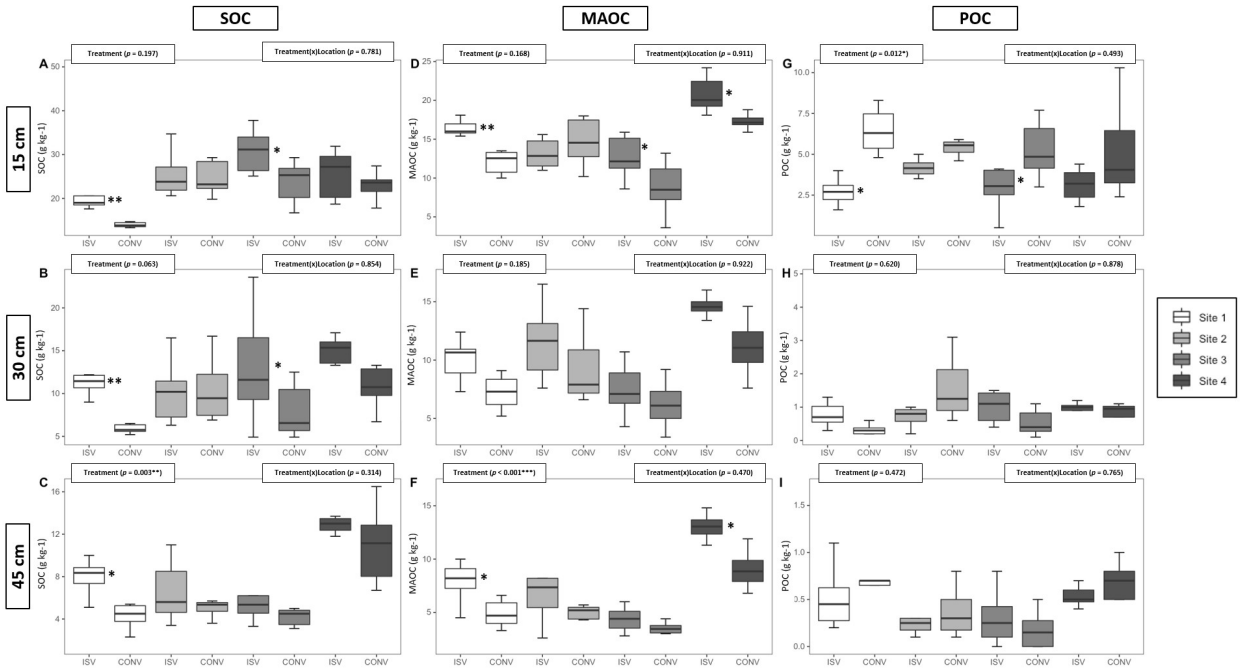
Figure 6: Soil carbon mineralization rates over a 35-day incubation from integrated sheep-vineyard (ISV) and conventional understory (CONV) managed soils



Soils were incubated and measured for cumulative C mineralization ($Cmin_{total}$; ug CO_2-C g $soil^{-1}$ day^{-1}) via microbial respiration rates at seven time points (1, 3, 7, 14, 21, 28, and 35 days) in soils from three depth zones (A) 0–15cm, (B) 15–30cm, and (C) 30–45cm. Soil C mineralization was also expressed relative to total SOC content ($Cmin_{soc}$; mg CO_2-C g SOC^{-1} day^{-1}) for depth zones (D) 0–15cm, (E) 15–30cm, and (F) 30–45cm. Treatment and treatment(x)location significance was calculated across sites (n=64).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

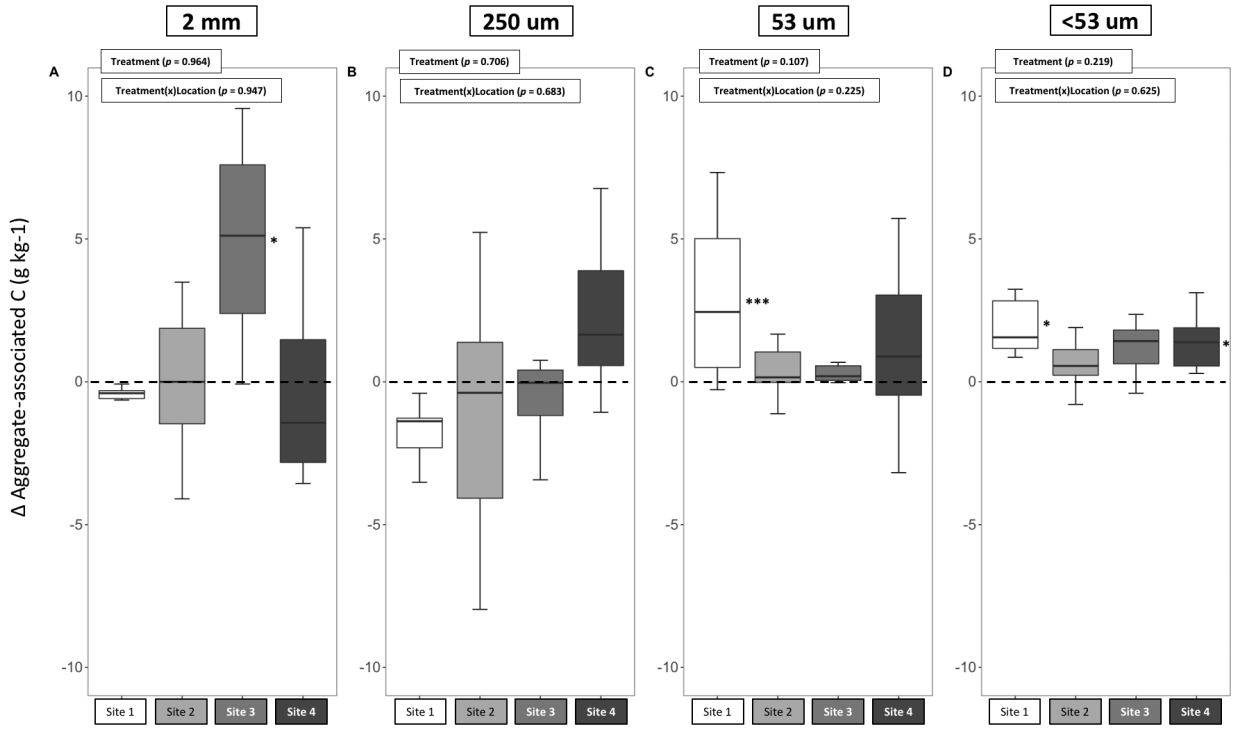
Figure 7: Soil carbon stabilization and storage pools in integrated sheep-vineyard (ISV) and conventional understory (CONV) managed soils



(A-C) Total soil organic C (SOC), (D-F) mineral-associated organic C (MAOC), and (G-I) particulate organic C (POC) were measured at three depths zones (0–15 cm, 15–30 cm, and 30–45 cm) from integrated sheep-vineyard (ISV) and conventional vineyard understory (CONV) managed soils. Shown are the treatment and treatment(x)location statistical significance across sites (n=64). For each location, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Error bars represent standard error. Asterisks (*) denote significant treatment differences at each depth increment.

* p <0.05; ** p <0.01; *** p < 0.001.

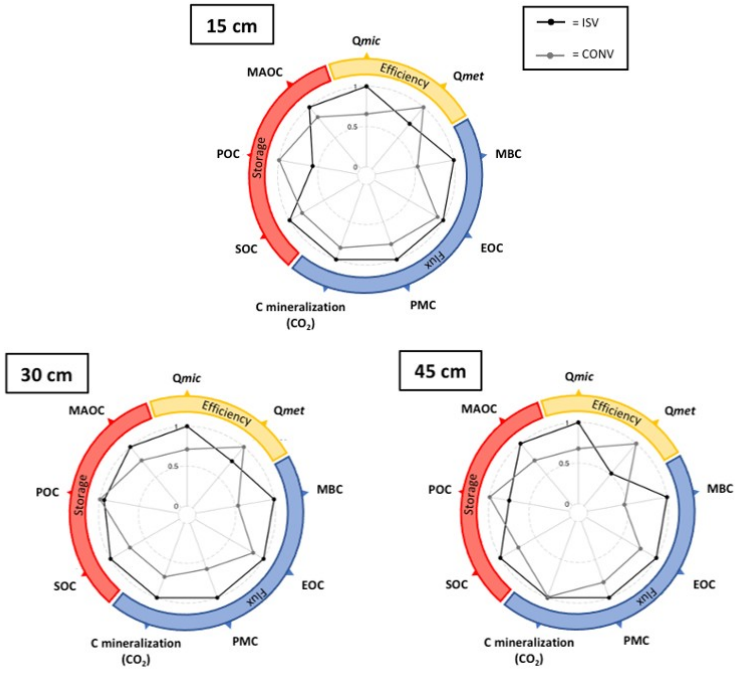
Figure 8: Aggregate-associated C pools in integrated sheep-vineyard (ISV) and conventional understory (CONV) managed surface soils (0–15 cm depth)



Surface soils (0–15 cm depth) were measured for the total C content associated with four soil aggregate physical size fractions (>2000um, 250-2000um, 53-250um, and <53um; A-D). For A-D, the dashed bar represents the CONV treatment mean for each paired site. Boxes above or below the line represent the relative increase or decrease in ISV values (Δ) for each site. Shown are the treatment and treatment(x)location statistical significance across sites (n=64). For each location, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Error bars represent standard error. Asterisks (*) denote significant treatment differences within site at each size fraction.

* p <0.05; ** p <0.01; *** p < 0.001.

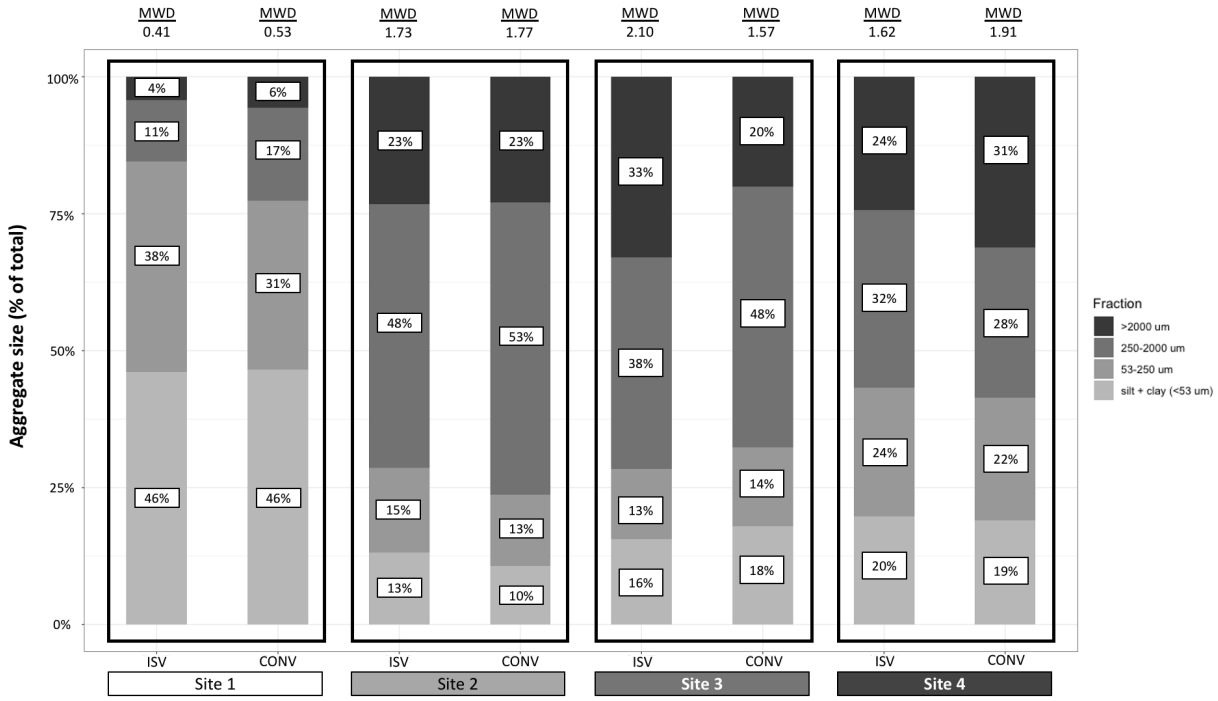
Figure 9: Proposed linkages between soil carbon flux and storage as impacted by perennial crop-livestock integration



Central radar plots with mean normalized values of soil carbon storage pools, flux pools, and microbial use-efficiency indicators from integrated sheep-vineyard (ISV) and conventional vineyard understory (CONV) managed soils at three depth zones (0–15, 15–30, and 30–45 cm).

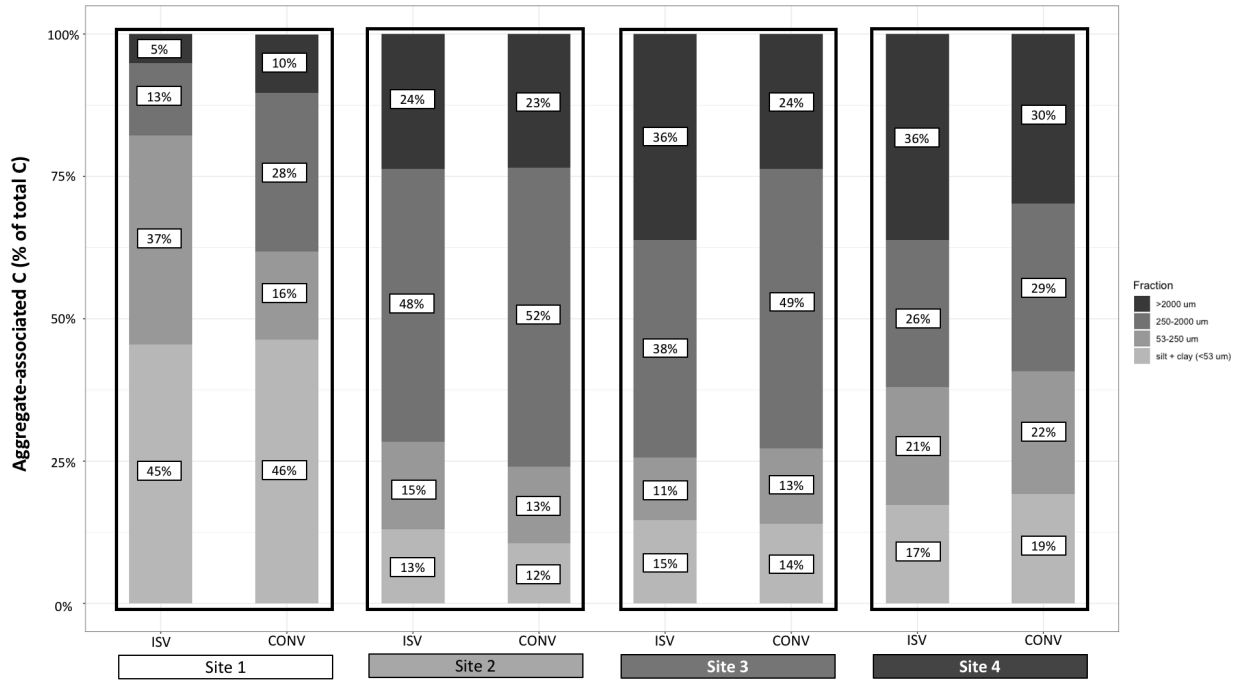
SUPPLEMENTAL TABLES AND FIGURES

Supplemental Figure 1: Aggregate size distribution from integrated sheep-vineyard (ISV) and conventional understory (CONV) managed surface soils (0–15 cm depth)



Relative distribution of four soil aggregate physical size fractions (>2000um, 250-2000um, 53-250um, and <53um). Shown are mean values for each size category and the mean weight diameter (MWD). For each site, a Tukey-Kramer means (n=16) comparison was used to evaluate significant pairwise difference between each treatment. Treatment and treatment(x)location significance was also calculated across sites (n=64). For MWD, both the Treatment (p=0.953) and treatment(x)location interaction (p=0.979) effects were non-significant. Treatment and treatment(x)location effects were also non-significant for all size fractions.

Supplemental Figure 2: Relative distribution of aggregate-associated C pools in integrated sheep-vineyard (ISV) and conventional understory (CONV) managed surface soils (0–15 cm depth)



The relative distribution (% of total C) across four surface soil (0–15 cm depth) aggregate physical size fractions (>2000um, 250-2000um, 53-250um, and <53um). For each site, a Tukey-Kramer means (n=16) comparison was used to evaluate significant difference between each treatment. Treatment and treatment(x)location significance was also calculated across sites (n=64). Treatment and treatment(x)location effects were non-significant for all size fractions.