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A stacked application of soil health building principles is key to enhancing soil ecosystem multi-functionality in a semi-arid almond agroecosystem

By

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THESIS

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in

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of the

UNIVERSITY OF CALIFORNIA

DAVIS

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Abstract

Identifying strategies to rebuild healthy, living soils is critical to not only ameliorating widespread soil degradation resultant from industrial agriculture, but importantly enhancing a multitude of soil ecosystem functions that are fundamental to an Agroecological transition. While many soil health building practices have gained traction, the benefits of these practices are highly dependent on cropping system, co-management practices, climate, and soil type. This is particularly true in California's almond agroecosystems where a combination of semi-arid climates, no-tillage management, and a historical emphasis on aboveground production components has left soils degraded and strategies to build healthy soil unclear. Therefore, this study tested the use of a regionally-specific survey approach in almond agroecosystems to evaluate relationships between soil health building principles underlying management and soil ecosystem multifunctionality. Specifically, the objectives of this study were to: 1) document the range of soil health building principles used in almond orchard management strategies; 2) measure physical, chemical, and biological indicators of soil ecosystem functioning associated with these strategies; 3) identify linkages between soil health building principles, measured soil indicators, and soil ecosystem functioning in perennial systems; and 4) reflect on the impacts of soil health and principally-based management strategies for more multifunctional agroecosystems.

The results of this study indicated that a diverse and stacked application of soil health building principles in practice may be the most effective strategy to enhance multiple soil ecosystem functions simultaneously. The highest performing cluster of orchards utilized animal grazing to manage diverse vegetative understories and had the highest soil organic carbon,

total nitrogen, ACE soil protein, available P, soil respiration, and robust and diverse soil

communities – resulting in the highest overall multifunctionality score. In contrast,

management strategies that reflected minimal soil health building principles (e.g., organic

amendments and/or winter living cover) clustered with the conventional management of this

region, bare soils that reflect no soil health principles in management. Although more research

is needed to investigate the capacity of popular management practices to meaningfully shift soil

ecosystem functional indicators in these agroecosystems, this study and its approach offers

new insights into the efficacy of a principally-based framework and analysis of management's

impact on soil ecosystems and their functions.

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1. Introduction

The industrial food paradigm calls for agriculture to prioritize crop yield as its primary function – with economies of scale, monocultures of few commodity crops, petrochemically-derived inputs, and mechanization as key to successfully attaining ever-increasing crop yield targets[1]. Although this model is often promoted as the only means to "feed the world", evidence continues to show that it comes with serious and broad negative externalities such as groundwater nitrate pollution[2,3], eutrophication[4], and loss of biodiversity[5–7] across the world. Further, these models of food production are neither the most productive[8,9] nor successful at feeding people in a sovereign[10–12] and ecologically[8,9] sound way. The negative externalities of industrial agriculture are also likely to be exacerbated as increasing evidence suggests a higher vulnerability to the biophysical[13–15] and socioeconomic[16] impacts of climate change and natural resource degradation. To address these negative externalities and build resilience to climate change, we must envision agricultural models that prioritize food production, human wellbeing, and environmental stewardship simultaneously.

A transition to Agroecological models may be the key to regenerating degraded landscapes and enhancing the multifunctionality of agriculture[17] to address the socio-ecological externalities of industrial food production. The design and management principles underlying Agroecological models can be applied across climatic and ecological contexts to lower reliance on external inputs by enhancing the internal regulation of biologically-mediated processes that drive agroecosystem functions[18–20] . Soil ecosystems play an essential role in Agroecological models because many of the biologically-mediated processes that drive agroecosystem functioning take place in the soil[21,22] – and therefore are soil ecosystem functions. However,

widespread use of industrial practices have reduced the soil's capacity to support ecosystem functions that underly sustainability and resilience such as soil carbon cycling, regulation of nutrient cycling, maintenance of soil structure, water storage, and conservation of soil biodiversity[23–26]. Therefore, management strategies need to restore healthy soil and center soil as a vital living ecosystem that can continually support a multitude of functions[25].

Rooted in the basic principles of Agroecological models, a principally-based approach to soil management – rather than a set of practices – is gaining traction as a way to build healthy soils that restore soil ecosystem functionality[19,25,27,28]. Soil health building principles include i) reduced and more strategic disturbances, ii) continuous soil cover, iii) consistent and diverse organic matter supply, iv) maximized living plant cover, and v) greater planned biodiversity. Although strategically applied disturbances play a necessary role in production – such as helping to liberate soil nutrients through microbial processing and preparing soil beds – reducing the extent of physical and chemical disturbances minimizes destruction of soil structure and disruptions to soil communities and their habitats[29]. Maintaining soil cover provides a habitat for more diverse biotic interactions while reducing potential abiotic disturbances that erode top soil and destroy soil structure[30]. Regular and continuous supplies of organic matter in diverse forms provide the energy and nutrients required by soil communities to perform biological processes[23]. Increasing the presence and continued maintenance of living plant cover supplies active deposits of organic matter directly into the soil ecosystem through rhizosphere exudation, root and shoot turnover, and above ground biomass accumulation[31,32]. The growth and turnover of living roots also creates a diversity of physical habitats, which contributes to more suitable conditions for active and diverse soil food webs[33]. Finally,

intentional increases in planned biodiversity, defined as the temporal and spatial diversity that results from management decisions[18,34], provides a range of habitats and resources to support soil food webs with co-benefits for conservation of aboveground associated biodiversity.

There are a wide range of management practices (e.g., no-tillage, cover cropping, mulches, organic amendments) that reflect both individual and stacked applications of soil health building principles. These strategies are gaining traction for their potential to increase soil organic carbon[35–37], improve soil structure[38,39], regulate nutrient cycling[37,40–42], and retain soil water[43,44]. Although these management practices have been shown to bolster soil ecosystem functionality, the impacts are inconsistent across climatic and edaphic contexts[38,43,45,46]. This may be partially explained by the fact that a large portion of the research aimed at identifying effective soil health building *practices*, and measuring their impacts on soil ecosystem functional outcomes, is conducted in temperate climates with relatively high organic matter soils[38,47–49]. In contrast to these conditions, many other production regions are characterized by semi-arid climates with low water availability, inherently low soil organic matter[50], and a high carbon loss potential[51,52]. Given that the potential to build healthy soil is highly influenced by climatic and edaphic characteristics[50], uncertainties remain around the translatability of increasingly popular soil health building practices across diverse environments. Therefore, devising context-specific soil health building strategies around principles rather than practices may be more effective at drawing links between management and soil ecosystem functional outcomes, especially in those regions that lack robust research reflecting environmental and cropping system characteristics.

A context specific approach may be particularly useful in California almond agroecosystems where semi-arid climates and heavy textured soils are met with research-informed industrial standards that emphasize aboveground production components to increase yields[53], manage pests , recycle byproducts, and optimize harvest operations to reduce dust[54]. A lack of emphasis on soils in these agroecosystems has resulted in widespread degradation and ubiquitous challenges such as poor soil structure[55], high soil salinity, decreased water infiltration, and high erosion and runoff potential[56,57]. Strides have been made to address the consequences of soil degradation in California almond agroecosystems through adoption of soil health building practices that reduce disturbance such as no-tillage[58,59]. Although reducing physical disturbances had been a successful in other regions[38,39], the conversion to no-tillage has been shown to have tradeoffs such as decreased water infiltration rates, increased compaction, and surface sealing in semi-arid almond agroecosystems like California's[44,60–62]. This is likely due to the fact that standard co-management practices maintain the low organic matter, clay-dominate soils bare[58] and exclude all other soil health building principles that are critical to functional soil ecosystems. While there are a broad range of management strategies that would introduce more fundamental soil health building principles and address potential tradeoffs of no-tillage, few studies have explored the impact of stacked management strategies on soil ecosystem functions in this particular agroecosystem.

Additional practices that reflect the other soil health building principles such as surface residue retention, winter and continuous living cover[37,43,63,64], inputs of organic amendments[62,65], and animal integration[42] have shown promise to improve soil ecosystem functions in Mediterranean orchard agroecosystems with similar climate characteristics as

California. However, key differences in standard co-management practices and study design make it difficult to infer outcomes for California almond production. First, studies exploring management practices that reflect additional soil health building principles often include tillage because physical disturbance remains a standard practice in many Mediterranean agroecosystems[63,64,66–68]. Given that tillage is not a standard practice in California almond orchards, the impacts of these additional management strategies in tandem with a no-tillage will likely differ. In addition, the duration of studies from the Mediterranean region were frequently designed to be short-term[30,62,64], leaving questions about the long-term impacts of management on soil ecosystem functions – especially for those with slower process rates such as accrual of stable soil organic carbon[23]. It was also uncommon for these studies to implement multifunctionality frameworks aimed at evaluating the impacts of a broad range of management strategies on the provision of multiple soil ecosystem functions. For example, some studies assess the impacts of individual management strategies on multiple functional outcomes[30,37,42,62,63,65,66,69] whereas some evaluate a range of management strategies on only few selected soil ecosystem functions[64]. This has left a gap in knowledge about the differing soil ecosystem functional synergies and tradeoffs that may result from long-term applications of individual principles (e.g., only input of organic amendments) or multiple stacked principles (e.g., combinations of continual living cover that increase organic inputs, soil cover, and increased planned biodiversity) in working landscapes.

A soil survey approach across a range of orchard management systems was used to address knowledge gaps around both the quantifiable benefits of multifunctional soil ecosystems and the context-specific strategies to build healthy soils in California perennial

agroecosystems. We aimed to better define healthy soil in almond agroecosystems by drawing

connections between soil health building principles that underlie management strategies and

measurable indicators that describe soil ecosystem functional outcomes. Specifically, the

objectives of this study were to: 1) document the range of soil health building principles used in

almond orchard management, 2) measure physical, chemical, biological indicators of soil

ecosystem functioning to 3) identify linkages between soil health building principles, soil

indicators, and soil ecosystem functioning in this agroecosystem and 4) reflect on the impacts

of soil health and principally-based management strategies for more multifunctional

agroecosystems.

2. Materials and methods

2.1 Agroecosystem characteristics & site selection:

The presence and extent of soil health building principles used in California's

commercial almond orchards was used to explore the relationships between management and

indicators of soil ecosystem functionality. The orchards selected for this study were located

within a 37-mile radius of Davis, CA (37.54° N, 121.76° W) to minimize variation in the inherent

climatic and edaphic characteristics that modulate soil health and ecosystem functionality[35,50].

Orchards were located on the western side of the Sacramento Valley and included sites in Yolo,

Colusa, and Solano Counties. This region of California has a Mediterranean climate with wet,

cool winters, dry, hot summers, 419 mm annual average precipitation, and 24.7° C maximum

and 8.6 C minimum annual temperature for the last two decades. During the sampling season

(Spring 2019), winter precipitation was 615 mm across the study region with mean maximum

temperatures of 23.5° C (2019) and mean minimum annual temperature of 9 °C

(https://cimis.water.ca.gov). Although Winter 2019 was considered a wet year, this region has been experiencing moderate to severe drought conditions throughout the 21st century (drought.gov/states/california). The soils in this region were predominantly silty clay, silty clay loam, and high clay content loams with a mixed alluvium parent material (http://websoilsurvey.sc.egov.usda.gov).

Almond agroecosystems were selected as the focus because this crop is ubiquitous across the California Central Valley landscape and represents the most acreage of any specialty crop. California almonds are currently ranked as the second highest grossing commodity and the largest contributor to the global commodity market, accounting for approximately 80% of the world's almond production[70]. The majority of almond ecosystems in the chosen study region were externally-regulated[53] with a strong emphasis on management practices that maximize tree productivity and input use-efficiency. For example, micro-irrigation systems are used to deliver external synthetic inputs and water directly to the tree root zone[58]. European honey bees are imported and seasonally leased to carry out pollination because large-scale almond monocultures lack the diverse resources and proximity to undisturbed habitats necessary to maintain sufficient populations of native pollinators[71]. In the study region, soils are typically maintained bare of vegetation using herbicides[58] to reduce the potential for competition between almond trees and other plants. No-tillage has been widely integrated into management and thus herbicides are commonly used to management vegetation. Bare soils also facilitate the dominant harvest practice of shaking and collecting almond kernels from the alleys, which requires little to no residues on the soil surface. It is becoming more common to allow some degree of resident vegetation during the winter when the tree is dormant.

During early winter 2019, a grower intake survey and site visits were conducted with UC

Cooperative Extension in the Sacramento Valley (Yolo, Colusa, and Solano Co.) to collect

information on broader management practices as well as specific soil health building strategies.

Twelve mature orchards were identified that fit within the soil textural and geographic

constraints and spanned a gradient of soil management strategies adopted for at least three

years. Soil texture was limited to the dominant classifications of the region and included silty

clay, silty clay loam, and loams with high clay content. Orchards that did not fall within these

classifications were not included in the study. Further, given that California hosts a wide range

of ecoregions, the geographic study area was restricted to within 30 miles of UC Davis in the

Central Valley ecoregion classification. Each of these orchards were detailed in terms of how

soil management translated to the five soil health building principles in both the center alley

and tree berm management zones **(Figure 1A)**. Orchards ranged from conventionally managed

bare soils **(Figure 1B**) to systems that exemplified multiple stacked soil health building

principles through maintenance of full soil cover with diverse plant communities **(Figure 1C)**

and continuously grazed silvopastures **(Figure 1D)**. All sites included in this study maintained

no-till management in their orchards – in line with most of the orchards within this study

region.

2.2 Soil sampling scheme and collection

Soil samples were collected in May 2019 from 12 orchards using a transect sampling

method. The month of May was chosen because it is the time at which soil management

strategies used during tree dormancy are adjusted to accommodate management needs during

the active growing season. The transects and starting sampling locations were established

within each orchard by reviewing soil maps and identifying locations that minimized variation in soil type within and across the 12 orchards (http://websoilsurvey.sc.egov.usda.go). Within each orchard, five replicate samples were taken along the transect (approximately 5 trees down and 1 row over) from two sampling zones (10 total samples) to capture the spatially distinct nature of almond productions **(Figure 1A)**. At each of the five locations along the transect three soils cores (0-20 cm depth) were retrieved and composited from the center of the alley (Alley) to form one replicate sample. Using the same protocol, composite samples were retrieved from the transition between the beginning of tree berm and edge of where alley management ends to capture where management may more directly impact the tree (Tree).

All soils were kept on ice during field sampling and transportation. Samples used for immediate analysis or extraction (within one week) were stored at 4° C, while a small subsample of approximately 4 g was separated and stored at -80° C for measurements performed at a later date. Two gram field-moist subsamples were used to quantify gravimetric water content by oven drying soils at 105° C and weighing them every 24 hours until constant weights were achieved[73]. Gravimetric water content was then used in procedures requiring dry weight equivalents for final calculations.

2.3 Soil ecosystem functions

A combination of commercial assessments (Cornell Assessment of Soil Health in Ithaca, NY) and in-house measurements were used to evaluate relationships between soil health building principles and multiple selected indicators for soil ecosystem functionality **(Table 1)**. The commercial lab assessments were used to quantify more commonly used indicators[26], while additional sensitive and novel indicators were quantified in house.

2.3A Building soil carbon

Several carbon measures indicative of different biological processes and carbon pools were selected as indicators of the soil's capacity to build carbon. Approximately 2 g of oven dried soil was ground using a ball mill and analyzed for Soil Organic Carbon (SOC) by a combustion method (Costech ESC 4010 Elemental Analyzer). On the same day that each orchard sampling event occurred, soil microbial biomass fumigation-extractions were performed on 6g of fresh field moist soil using chloroform fumigation extraction methods[74]. The fumigated and unfumigated extracts were analyzed for extractable organic carbon content (Vario Cube, Elementar). Soil Microbial Biomass Carbon (MBC) was calculated as the difference in extractable organic carbon content between fumigated and unfumigated samples, using a correction factor of 0.35[74]. Permanganate Oxidizable Carbon (POxC) was quantified via colorimetry by reacting approximately 2.5g of air dried soil sample in solution with 0.2 M Potassium Permanganate[75].

2.3B Supplying fertility

Total soil Nitrogen (TN) was quantified by analyzing approximately 2g of oven-dried ground soil using a combustion method (Costech ESC 4010 Elemental Analzyer). Measures of Autoclaved Citrate Extractable (ACE) soil protein – a proposed indicator of organically bound N potentially available for microbial processes[76,77] – were quantified by recording absorbances from colorimetric assays of sodium citrate soil solutions[78]. Phosphorus (P) and potassium (K) were extracted using a Modified Morgan's solution[78]. The extracts were then analyzed using an Alpkem Automated Rapid Flow Analyzer for available P and Inductively Coupled Plasma Emission Spectroscometry for available K.

2.3C Improving soil structure

Intact soil cores were used exclusively to determine soil bulk density from each sampling location. Soil bulk density (BD) was determined by drying the contents of each soil core at 105° C until constant weight, and then dividing the oven dry soil weight by the volume of the soil core[72]. Wet Aggregate Stability (WAS) was determined by using a putting soil samples in the Tyler Coarse Sieve Shaker and subjecting them to a rain simulation procedure[79]. The slacked soil material collected during the rain simulation and the remaining intact aggregates were oven dried. Wet aggregate stability of each soil sample was calculated as the portion of soil stable aggregates that remained intact after the rain simulation procedure.

2.3D Supplying soil water

Available Water-holding Capacity (AWC) was determined by measuring the water stored in each soil sample between field capacity and wilting point[78,80]. Briefly, two ceramic plates were filled with a soil sample and placed in two separated chambers that reached pressures for field capacity (10 kPa) and wilting point (1500 kPa). Soil sample weights were recorded once the samples were allowed to equilibrate to the target pressure. The available water capacity was calculated as the soil water content contained between the point of field capacity and wilting point.

2.3E Supporting diverse and active soil communities

Phospholipid fatty acid (PLFA, Ward Laboratories, Inc.) and nematode food web analyses were conducted to describe multiple characteristics of the size and structure of orchard soil communities. PLFA biomarkers were used to quantify the total fungal biomass. Characteristics of the nematode communities were used as indicators of soil food web condition because they

integrate changes in microbial populations[81] and also respond to management changes such as amendment addition or disturbance[82]. Nematodes were extracted from 200 cm^3 of field moist soil using a sieving and decanting technique followed by purification on a glass Baermann funnel for 48 hrs[83]. The total number of nematodes in each sample solution were counted, and the first 200 encountered on each slide were identified to the genus level[84]. An exception was made for those nematodes in the families *Qudsianematidae* and *Tylenchidae* because the genera within these groups are difficult to distinguish morphologically. Nematode taxa abundance was used to estimate total nematode biomass and calculate metabolic footprints representing the contribution of different functional guilds of nematodes to ecosystem functions based on their size-dependent metabolic activity[85]. Calculations of nematode metabolic footprints and estimations of nematode biomass were completed using the online platform, NINJA: 'Nematode INdicator Joint Analysis'[86].

In addition to community characteristics, soil respiration (RESP) was measured as the carbon dioxide (CO_2) released over the span of a 4 day incubation[78]. Air dried soil samples were rewetted by allowing each sample to draw water up via capillary action from a rewetting basin[87]. Rewetted samples were placed in sealed chambers containing a potassium hydroxide (KOH) alkali trap to collect CO_2 throughout the duration of the incubation[88]. Soil respiration was calculated by subtracting the amount of CO_2 in trap solution from the amount of CO_2 in trap solution from a control chamber with no soil.

2.4 Statistical analyses

All data analysis was performed in R (version 3.6.1). Prior to analysis, the distribution of data for each indicator was examined for normality. Data for indicators that required

normalization had positive skewness and were transformed to address abnormal distribution prior to further data analysis. Principal component analysis (PCA) was used to visualize patterns and underlying structure in relationships between the measured soil indicators and orchard soil health building principles. All PCAs were performed using the vegan package in R[89].

A k-means partitioning methodology was used to identify the optimal number of orchard clusters for both of the scaled Alley and Tree datasets based on indicators of intra-cluster homogeneity and inter-cluster heterogeneity[90]. Scree plots were used to identify an initial range of k values for clusters that minimized sums of square error using the "elbow" method (**Figure S1**)[91]. Silhouette and Gap statistics were calculated for each k cluster to determine the optimal number of clusters for the Alley and Tree datasets[90]. First, a silhouette statistic – which measures how similar datapoints within a cluster are to each other – was calculated for each k value (e.g., k = 3, 4, 5 clusters) and evaluated to identify which amount of clusters maximized intra-cluster homogeneity[90]. A gap statistic was also quantified to evaluate inter-cluster heterogeneity by assessing where the maximum distance occurs between clusters using the elbow method[92]. All cluster analyses were performed using a combination of the Stats, Cluster, and facttoextra packages[93,94].

Linear mixed-effects models were used to measure and compare indicators of soil ecosystem functions for each cluster. Appropriate models were selected by comparing Akaike Information Criteria (AIC) scores and pairwise comparisons of each model. Models were fit with a fixed effect for "cluster" and a random effect for "site". Post-hoc Tukey HSD's pairwise comparisons were used to assess differences between each cluster. Mean values of the indicators for the fixed effect were considered significantly different if the p-value < 0.05, and a

trend was a *p*-value < 0.1[95,96]. For the abundance counts of individual nematode genera,

assumptions of normality and homogeneity of variance could not be met even with

transformation, so differences between clusters were assessed using non-parametric Kruskal-

Wallace tests followed by post-hoc Dunn's tests.

Radar plots were generated by scaling each indicator from 0 to 1 and then plotting the

scaled mean value of each indicator by cluster using the ggradar package in R.

Multifunctionality indices were calculated using the averaging approach where each individual

indicator was standardized using a "more is more" or "less is more" (e.g., soil bulk density)

method [97,98]. The standardized indicators were averaged for each of the soil ecosystem

functions and then summed into a single multifunctionality value where each of the five

functions were weighted equally.

3. Results

3.1 Soil health principles in practice

The 12 orchards included in the study reflected a range of acreages, production goals,

and broader management systems – leading to a wide representation of individual and stacked

applications of the five soil health principles (**Figure 1E**). There were minimal physical

disturbances documented given the predominance of no-tillage practices in almond

agroecosystems from this study region. Physical disturbances in orchards with animal

integration occurred due to the physical impacts of trampling and hoof action during grazing

events.

Strategies to increase the presence of living plant cover in almond orchards were

differentiated based on the duration. Plant living covers such as cover crops (sites 4, 9, 10) or

resident vegetation (sites 5, 12) were grown for the duration of the winter, while the tree is dormant, and terminated in early spring to allow time for decomposition of plant residues in preparation for kernel harvest. Multiple orchards included in this study adapted traditional kernel harvest methods and continued irrigation into the active growing season to maintain continuous plant living covers (sites 3, 7, 11).

Plant residues were the main constituent of soil covers present in the surveyed almond orchards. The extent of soil coverage documented in each orchard was predominantly a function of spatial location of plants, duration of growth, and termination strategy. Orchards were considered to have bare soils if management aimed to eliminate any coverage of the soil surface. While some sites applied soil health building principles in Alley management, many managed tree berms to maintain bare soil (sites 4, 5, 8, 10, 11, 12). Partial soil cover was noted in orchards where plant residues provided cover for a portion of the year (sites 4, 5, 9, 10, 12) – which occurred in orchards where winter plant living covers were terminated and residues were left to decompose so bare soils were present to facilitate kernel harvest. Finally, orchards were considered to have full soil cover if management aimed to leave no periods of the soil surface bare (sites 3, 7, and 11).

A range of amounts and types of organic matter inputs were documented. Organic amendments in the form of compost (site 4, 9) or recycled almond hulls and shells (site 9) were broadcasted across the soil surface in multiple orchards but not incorporated. Management that included any type of plant living cover were considered to increase inputs of organic matter through rhizodeposition, root turnover, and growth of aboveground plant biomass. While animals do remove energy (carbon) and nutrients during metabolism, the conversion of

plant materials and deposition of animal manure and urine were considered diversified inputs

of organic matter in orchards with animal integration (sites 3, 7, 9).

Multiple strategies were implemented in the surveyed almond orchards to increase

planned biodiversity. Plant communities ranged from resident vegetation (sites 5, 7, 11, 12)

and established forage pastures (sites 3) to multi-species planted cover crop mixtures of

brassicas, legumes, and/or grasses (sites 9, 10, 11). Ruminant and non-ruminant animals were

also integrated to manage and then terminate living cover during the winter (site 9) or used in

an extensive rotational model where animals were continuously moved across an orchard in

small paddocks (sites 3, 7). In addition to diversifying the types of organic matter inputs

entering the soil, the ruminant and non-ruminant animals documented in multiple orchards

could also contribute to increases in the spatial and temporal diversity of plant communities

through grazing activities.

3.2 Orchard clustering by soil health building principles

Principal components analysis (PCA) of the Alley and Tree indicated strong relationships

between soil ecosystem functional indicators as well as a differentiation between orchards

along the first two principal components; which together accounted for 54.2% and 56.7% of the

variability in the Alley and Tree respectively (**Figure 2A-B**). Indicators associated with building

soil carbon, supplying soil fertility, and supporting soil communities trended together and

loaded along the first principal component, which accounted for 41.6% (Alley) and 46.2% (Tree)

of the variability. Indicators with the strongest contributions to PC1 for the Alley included SOC,

POxC, TN, and RESP - while additional biological indicators such as nematode and fungal

biomass, nematode footprints, and MBC contributed to PC1 for the Tree. Soil bulk density

loaded along PC1 and strongly grouped multiple orchards in both the Alley and Tree

management zones, indicating higher mean values of soil bulk density and more compaction at

these sites **(Table S1)**. The second PC accounted for a lower portion of the variability – 12.6% in

the Alley and 10.5% in the Tree. Indicators with the strongest contribution to PC2 in the Alley

included AWC and WAS. In the Tree K also trended along PC2.

Three main orchard clusters were identified for both the Alley and Tree using a k-means

cluster analysis method of the measured indicators of soil ecosystem functions. (**Figure 2C-D**).

Cluster A (sites 1, 2, 4, 6, 8, 10) grouped the largest number of sites and included orchards with

bare soils that reflected no applications of soil health building principles as well as orchards

with a combination of winter plant living covers and/or organic matter inputs. The living covers

documented in orchards from cluster A were planted cover crops while the organic matter

inputs included compost or recycled almond byproducts. Three sites were grouped into cluster

B (sites 5, 11, 12) and included one orchard with a continuous living cover of reseeded cover

crops and resident vegetation. The other two sites from cluster B had resident vegetation only

in the winter. Finally, cluster C (sites 3, 7, 9) grouped the three orchards with animal integration

and a stacked application of soil health building principles including winter or continuous living

covers, partial or full soil cover, a range of organic matter inputs, and high levels of planned

biodiversity.

While soil textural criteria were used to minimize variation within and across orchards

for soil sampling in this study, small variations in sand, silt, and clay content can have notable

impacts on measured indicators – and thus could affect cluster analyses aimed to explore

management impacts. However, there were no clear groupings based on soil textural

classifications for each orchard cluster (**Figure 1E**) and no significant differences in clay content across the Alley and Tree (P > 0.1; data not shown).

3.3 Soil ecosystem functional outcomes

3.3A Improving soil structure

Orchards in Cluster C had significantly lower mean soil bulk density, and thus lower soil compaction, than cluster A (P = 0.050) in the Alley, while cluster B (P = 0.682) had an intermediate, non-significant mean value **(Table 2).** Although regressions for the Tree zone did not meet the threshold of significant difference, similar trends for soil bulk density were identified across the three orchard clusters. There were no significant differences in wet aggregate stability between the three orchard clusters.

3.3B Supplying soil water

The three orchard clusters did not have significantly different water holding capacities (g water/g soil) in both the Alley and Tree zones.

3.3C Building soil carbon

Strong significant differences were found across orchard clusters in the Alley and Tree for multiple indicators of building soil carbon (**Table 2**). In the Alley, mean values of soil organic carbon (g C/kg dry soil) were higher in cluster B (P = 0.002) and C (P < 0.001) than in cluster A, but they did not differ from each other (P = 0.145). However, soil organic carbon was different across all three orchard clusters in the Tree zone, with cluster C having 2.9 and 1.9 times higher mean values compared to cluster A (P < 0.001) and B (P = 0.005), respectively. Significant differences between orchard clusters in the mean values for microbial biomass carbon (ug C/g soil) were found in the Tree zone only; with Cluster C having higher biomass than Cluster A (P =

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0.002) and an intermediate, non-significant difference with Cluster B (P = 0.209). The mean value of POxC (ppm) for cluster C was more than double that of Cluster A in both the Alley and Tree zones (both P < 0.001). While Cluster A and Cluster B did not differ in the Tree (P = 0.215), POxC was 1.5 times higher for Cluster B compared to Cluster A in the Alley (P = 0.002).

3.3D Supplying soil fertility

Cluster C had approximately three times higher mean values of ACE soil protein than that of both cluster A and B in the Alley and Tree (all P < 0.001;**Table 2**). However, Cluster A and B did not differ from each other in their mean values for ACE soil protein across the Alley (P = 0.739) or Tree (P = 0.863). In the Alley, total nitrogen was different across all three orchard clusters with cluster C having 2 times more nitrogen than cluster A (P < 0.001). Similarly, in the Tree zone cluster C had an approximately 1.9 times higher mean value of total nitrogen than cluster A and B (P = 0.001 and P = 0.050, respectively), but A and B didn't differ from each other (P = 0.305). Available P (ppm) followed similar trends where Cluster C had 13 and 3.4 times higher mean value of available P than both cluster A (P < 0.001) and B (P = 0.012) in the Tree. In the Alley cluster A had lower mean values for available P than cluster B (P = 0.007) and C (P < 0.001), which did not differ from each other (P =0.223). Finally, orchard clusters did not differ in available K across both the Alley and Tree zones.

3.3E Supporting diverse and active soil communities

Orchard clusters showed distinct soil biological profiles. First, there were differing levels of soil respiration, an indicator of microbial and soil fauna activity (**Table 2**). In the Alley, cluster C had average levels of soil respiration two times higher than that cluster A (P = 0.012), with cluster B having intermediate values. A similar trend was seen in the Tree, with cluster C having

two times higher soil respiration than both cluster A (P < 0.001) or B (P = 0.001). When fungal biomass was measured by PLFA there were no significant differences between clusters in the Alley, but a trend for cluster C to be numerically higher than A in the Tree zone (P =0.064).

Nematode food webs showed dramatic differences in ecological indicators and community composition between clusters (**Table 2**). Cluster C hosted levels of nematode biomass more than seven times higher than cluster A in the Alley (P = 0.026) and nine times higher in the Tree (P = 0.001). These increases in nematode biomass were spread across the food web. Cluster C had 3 and 2.5 times higher mean values for the nematode fungivore footprint than cluster A and B (both P < 0.05) in the Tree. Bacterial feeding nematodes also responded to applications of soil health principles, as seen by higher levels of the nematode bacterial metabolic footprint in cluster C compared to A across the Alley (P = 0.013) and Tree (P = 0.006). Specifically, this was driven by greater abundances of bacterial feeding nematodes in the genera *Panagrolaimus*, *Rhabditis* and *Prismatolaimus* (*data not shown;* P < 0.05). Trend differences were also seen in bacterial feeding nematode abundance between cluster B and cluster A with *Rhabditis* being more abundant in B compared to A (P = 0.01) in both locations. The metabolic footprint for predatory nematodes were highest in cluster C (all P < 0.05), likely due to greater abundances of the large *Mylonchulus* (P = 0.01).

Similarly to bacterial feeders and predators, orchards in cluster C supported greater biomass and abundance of root herbivore nematodes, as indicated by the herbivore footprint (**Table 2**, P < 0.05). This trend was mostly due to high quantities of minor root tip feeding plant parasites such as Tylenchidae and *Tylenchorhynchus* at both locations (P < 0.05). However, more potentially serious pests such as *Pratylenchus* (root lesion nematode) were also more

abundant in cluster C compared to A in the Alley (P = 0.02) and compared to both cluster A and B in the Tree (P < 0.01). Contrastingly, though, the plant parasite, *Helicotylenchus*, was most abundant in cluster A than in cluster B orchards at the Tree (P < 0.01).

3.4 Soil ecosystem multi-functionality

Clear patterns in overall soil ecosystem functional outcomes by orchard cluster emerged from normalized data, with some differences evident between the Alley and Tree (**Figure 3A-B**). Across both management zones, cluster A – which included orchards with bare soil, organic matter inputs only, or winter living cover with organic inputs – consistently had the lowest values of measured indicators, excluding WAS. This translated to cluster A having the lowest Multifunctionality index in both the Alley (1.67 $\pm$ 0.08) and Tree (1.48 $\pm$ 0.10) (**Figure 3C-D**). Cluster C had the highest multifunctionality index of the three clusters in both the Alley (2.91 $\pm$ 0.15) and Tree (2.78 $\pm$ 0.18) and was significantly higher than cluster A across both management zones (Alley: P = 0.022; Tree: P = 0.011). However, cluster C was not significantly different than cluster B across both management zones (Alley: P = 0.954; Tree: P = 0.206), which had intermediate multifunctionality indices for the Alley (2.53 $\pm$ 0.104) and Tree (2.07 $\pm$ 1.04). Although there were no differences between cluster B and C, cluster B was only different from A in the Alley (P = 0.044).

4. Discussion

4.1 Stacking principles promotes higher soil ecosystem multifunctionality

Soils can support a multitude of agroecosystem functions that are essential to addressing the consequences of widespread soil degradation[25,26] and transitioning to a more ecologically sound, sovereign, and resilient Agroecological model of agriculture[18,27]. While there

is increasing evidence that management can improve essential functions such as building soil organic carbon[35–37], improving soil structure[38,39], regulating nutrient cycling[37,40–42], and retaining soil water[43,44], much of this work has been focused in temperate climates with relatively high soil organic matter levels. It may thus be both useful and necessary to explore how these management strategies – and their measurable impacts of soils ecosystem functions – translate to other environments such as those agroecosystems with semi-arid climates[99], clay-dominant soils[50], and regionally-specific management systems[58]. Using a regionally-specific survey approach, this study explored the relationships between soil health building principles underlying California almond agroecosystem management, multiple soil ecosystem functions, and multifunctionality.

Results show that a diverse and stacked application of soil health building principles is likely essential to enhancing multifunctionality in this agroecosystem context. Soil ecosystems with applications of single or few principles without prioritizing long plant living covers and high levels of planned biodiversity did not differentiate from bare soils and had the lowest multifunctionally score in both the Alley and Tree (cluster A). In contrast, orchards with the most Agroecological approach to management (those with many stacked principles) clustered together and presented the highest levels of soil ecological functioning (cluster C) – especially when animals were integrated and in the Tree zone where the majority of crop root growth and activity takes place[100]. This result is in line with other studies that find a stacked application of practices reflecting diverse principles can produce synergistic effects for soil ecosystem functioning[57,98]. However, while multifunctionality scores can be useful for exploring high level relationships between land use, agricultural management, and ecosystem outcomes[101–103], it is

also important to evaluate the relationships between individual soil ecosystem functions and management. This is especially the case in the current study as results from each soil ecosystem function provide interesting insights into the benefits, tradeoffs, synergies, and nuances of management in this semi-arid perennial agroecosystem.

4.2 Strategies to support diverse and active soil communities

Given that soil communities are essential to enhancing soil ecosystem functional outcomes[21,102,104,105], the differences observed in indicators of food web compositions across the three orchard clusters suggest that some applications of soil health building principles may be more effective than others at supporting active and diverse soil communities. Notably, cluster C – the grouping of orchards with animal integration – had higher total nematode biomass across the Alley and Tree management zones than cluster A and cluster B. Nematode communities are highly responsive to management because of their biological characterstics[106–109], and have been shown to be sensitive indicators of ecological shifts because they play an essential role in regulating soil biological processes[110] and connect trophic levels across the soil food web[85]. The results from this study are in line with some past research in semi-arid agroecosystems that suggest external applications of organic matter with or without living covers may not be sufficient to increase total biomass of the nematode community[108,109,111]. Our findings show that soil ecosystems that received organic inputs alone or in combination with winter plant living cover did not differentiate from bare soils (cluster A) and had the lowest nematode biomass. While there are distinct agroecosystem differences, studies from semi-arid grasslands have found that extensive grazing can modestly increase soil nematode biomass[112–114]. This may be partially due to animal grazing's role in stimulating the basal resources of the

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food web that serve as prey for many nematode functional guilds[114]. Taken together, animal grazing with best practices could be a strategy that both reflects and facilitates a set of stacked soil health building principles that is essential for bolstering total nematode biomass in almond Agroecosystems.

While total nematode biomass may be indicative of the overall quantity of resources available to soil nematodes, nematode metabolic footprints give a more nuanced picture of how management influences food web structure, particular functional guilds, and the presence of food sources such as bacteria and fungi[85]. The metabolic footprints of bacterial and fungal feeding nematodes were highest in cluster C. This is perhaps due to the diversification and increased extent of basal resources provided by animal grazing via root turnover, rhizodeposition, increased plant primary productivity, and input of animal excreta that could support a wide range of microbes that serve as food sources[114,115]. The impact of animal grazing on basal resources and lower-level soil nematodes may have cascading effects unto higher tropic organisms of the soil food web[114] as the metabolic footprint for predatory nematodes in cluster C was higher than both A and B across the Alley and Tree. Such complex soil food web interactions have practical applications for crop production as they significantly influence the mineralization of nutrients like nitrogen[21,116,117]. Therefore, the presence and activity of these nematodes may be essential to the internal regulation of crop nutrient availability and soil fertility in these agroecosystems.

The orchards from cluster C also had the highest metabolic footprint for root herbivorous nematodes. While many of these herbivorous nematodes may not pose a threat to crop health, cluster C orchards had the highest quantities for minor root feeding parasitic nematodes

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Tylenchidae and *Tylenchorhynchus* as well as more serious pest threats like *Pratylenchus* across

the Alley and Tree. Crop parasitism by these nematodes can cause serious damage to plant

health[118] – and ultimately imparts a significant financial cost onto the agricultural sector

through lost productivity and chemical pest management[119,120]. The diversity and continuity of

the plant living covers in the orchards with animal integration (cluster C) may provide a partial

explanation as this management style could stimulate the growth of herbivorous soil

nematodes by providing high levels of diverse primary resources and a range of physical

habitats[118]. Further, the orchards from cluster C were all certified organic and must use

biological control for plant parasitic nematodes instead of chemical fumigation – which has

been documented to be a challenge for organic productions[121,122]. Although out of the scope of

this study, it is possible that hosting a diversity of predatory soil organisms, including

nematodes, may serve as a biocontrol – which could regulate populations of root feeding

nematodes to reduce the potential for crop damage[119,123]. In this particular agroecosystem,

animal grazing may help to bolster the resources and habitats necessary to maintain more

complex populations that could ultimately support biocontrol functions in the soil. However,

much more research about the impacts of animal grazing on soil food web structure more

broadly is needed. Further, studies exploring the potential relationships between animal

grazing, soil food web structure, and biocontrol of plant root parasitic nematodes would be

meaningful work – especially for those farmers that do not use chemical controls.

4.3 Stacked principles are key to building soil carbon

Soil carbon is the backbone of a healthy, living soil[23,124] and, therefore, identifying soil

health building strategies that (re)build this fundamental element of soil is essential to

enhancing soil ecosystem multifunctionality[125]. The results from this study suggest that there

may be effective strategies to build soil organic carbon in California almond Agroecosystems,

but that it will likely necessitate a diverse set of stacked soil health building principles in

practice. Orchards that exemplified the maximum application of principles in this study (cluster

C) had 2 (Alley) and 2.9 times (Tree) higher soil organic carbon than the lowest grouping of

orchards in cluster A – which included orchards with bare soils as well as orchards that applied

organic amendment with or without living cover. In contrast to our findings, meta-analyses

from similar semi-arid perennial agroecosystems have suggested that organic amendments may

be an effective way to build soil organic carbon[59,62,126]. Findings from these same meta-analyses

and additional studies have also found, while variable, neutral to positive impacts of organic

amendments paired with a living cover[59,108,126,127]. Although the findings from our study

contradicted this body of work, there may be multiple reasons that help elucidate these results.

First, many of the agroecosystems from past research also utilize tillage as a co-

management practice[68] – which may provide a pathway of incorporation for organic inputs into

the soil profile. In contrast, orchards from cluster A applied organic amendments using a

broadcast method that retained inputs at the soil surface, potentially making them susceptible

to erosive forces (E.g., rain and wind). In addition, persistent drought conditions across

California[99] – excluding the anomalous year when this study took place – have made it difficult

to grow robust living covers that supply high carbon inputs without additional irrigation and

may hamper the infiltration of dissolved organic solutes from broadcasted organic amendments

[128,129]. It's also plausible that the pulse of organic matter inputs – taking the form of

amendment applications and plant residue – in orchards from cluster A may be contributing to

a net soil carbon priming effect in these conditions[31,32,130–133]. Orchards from cluster A do principally reflect increased inputs of organic matter, however, the application of these inputs were in large and discontinuous pulses of carbon through annual amendment applications or winter living cover growth. These large pulse applications of organic matter inputs can result in overstimulation of soil microbial communities[134,135], and ultimately promote mineralization of endogenous soil organic carbon if there are not enough resources to sustain the population[136,137]. And this may be especially true within California's semi-arid agroecosystems, which are generally characterized by low values of inherent soil organic carbon[50] and high soil carbon loss potentials[51,52]. While these strategies do reflect applied soil health building principles, their effectiveness in practice is likely influenced by both inherent environmental contexts like soil texture and climate[138] as well as agronomic decisions such as plant community composition, amendment type, application method, duration of practice, and tillage[46]. Given this, additional research will be needed to build a more definitive understanding of the capacity for these particular strategies to build soil organic carbon in California almond agroecosystems.

Orchards grouped into cluster C both reflected a diverse and stacked application of soil health building principles and translated to the highest mean value of soil organic carbon across the Alley and Tree. The results from this study are also consistent with other findings that integrated crop livestock (ICL) systems can increase soil organic carbon across agroecosystems[139–143], including semi-arid climates[144–147]. ICL systems – including all the orchards that comprised cluster C in this study – may be effective at building soil carbon as they alter multiple fundamental biogeochemical pathways that can ultimately promote soil carbon accumulation. In particular, by transforming recalcitrant aboveground plant residues into more

labile, nutrient-rich animal dung and urnie[144] – which can more easily enter the soil

profile[128,129,148,149] and be assimilated for microbial processing[142,145,146,152–156] – grazing may

facilitate a more direct pathway toward long-term, stable soil organic carbon

accumulation[144,157–159]. This may also help to explain why labile carbon (POxC) was higher in

cluster C compared to the other clusters across the Alley and Tree. Animal grazing has also been

shown to stimulate increased root biomass growth[159–161] and rhizodeposition of labile carbon

compounds[151], which could further contribute essential resources to microbial processing of

soil organic carbon. In addition to ways that grazing directly contributes to building soil organic

carbon, ICL management requires the adoption of co-management practices that reflect diverse

and stacked applications of soil health building principles such as partial to continuous plant

living covers and biodiverse forage to meet animal dietary requirements[162]. While this study

adds to the increasing body of work that demonstrates the beneficial contributions of animals

to (re)building soil organic carbon, more research is needed to identify best management

practices in semi-arid agroecosystems, explore underlying mechanisms, and validate ICL

systems' potential contributions to soil organic carbon accumulation.

4.4 Animal integration as a tool to supply soil fertility

Although total global synthetic nutrient use has steadily increased over the last

century[163], there is an urgent need to transition toward a production model that meets crop

nutrient requirements without perpetuating fresh water pollution[2–4] and relying on external

inputs generated with finite fossil fuels[24,164]. Identifying management strategies that enhance

the internal regulation of nutrient cycling and availability is, therefore, a foundational soil

ecosystem function and paramount to meeting broader sustainability goals[24,165,166]. This study

found significant differences in multiple indicators of soil fertility across the orchard clusters. Most notably, cluster C had the highest mean value across both the Alley and Tree of multiple indicators of the soil capacity to supply essential nutrients compared to both cluster A and B, which were frequently insignificantly different from each other.

While these results add to broader research that demonstrates there are effective strategies to internally regulate nutrient cycles and supply soil fertility while reducing potential negative exteranlities[40,42,47,101,167], our findings also contradict the efficacy of particular soil health building strategies. First, the clustering of orchards that maintained bare soil with orchards that applied organic amendments with or without living cover (cluster A) is a noteworthy finding because it contradicts findings from similar crops in semi-arid environments that found these strategies increased total soil nitrogen[62,126]. This finding may have a practical explanation – similar to that of the SOC results – as the organic amendments applied in cluster A orchards were broadcasted across the soil surface and thus potentially subject to erosive forces. Further, if the application of organic matter in these orchards is causing a net soil organic carbon priming effect – as presented with SOC – then there is likely also net nitrogen mineralization taking place[168], making soil nitrogen suspectable to loss pathways such as leaching or denitrification[166]. In the case of the orchard from cluster A that planted a living cover with organic amendment applications, the low total nitrogen may also be a product of the cover crop species selection, which only contained grasses. In contrast to plant communities with leguminous species that increase soil nitrogen through biological nitrogen fixation[169], grasses can help retain soil nutrients through biomass accumulation but do not directly contribute to building soil nitrogen[37,170]. Plant community composition may help to

explain the marginally higher total soil nitrogen value in cluster B, which included orchards that had mixed plant communities that contained resident legumes like clover. One meta-analysis of studies from semi-arid, Mediterranean climates found that leguminous plant communities had positive impacts on total soil nitrogen, while mixed plant communities had variable positive to negative impacts[37]. Although the resident vegetation present in both the winter and continuous living covers of cluster B orchards included a mix of grass, broadleaf, and leguminous species, the untargeted nature of this strategy may not be sufficient to build substantial amounts of soil nitrogen over time in these agroecosystems.

Results from our study suggest that ICL systems, which reflect a diverse and stacked application soil health building principles, may be an effective strategy for building both total and potentially available supplies of essential soil nutrients. Orchards that utilized animal integration (cluster C) had more total soil nitrogen than the orchards from cluster A and cluster B . While research into the impacts of ICL systems on soil fertility dynamics is highly dependent on climate, soil type, and ICL design and management characteristics[145,171], recent research from pasture and no-till perennial agroecosystems in similar climates corroborate the findings from this study[142,160,172]. There may be multiple above- and belowground processes taking place that help to explain these higher total soil nitrogen values. Aboveground, moderate animal grazing can stimulate plant productivity and promote higher biomass accumulation[115,173,174] than ungrazed systems – potentially compensating for energy and nutrient removal that results from animal gains[175]. If these plant communities also comprise diverse functionalities such as leguminous species, which are a component of balanced ruminant nutrition[176], there may be a significant potential to build soil nitrogen with best grazing practices. The nitrogen that

comprises animal excreta is also returned to the soil in labile forms[177–179] that belowground soil communities can more easily assimilate[180,181], making the biological processes that regulate nutrient (re)cycling and organic matter accumulation more efficient than if nutrients were returned in more recalcitrant forms[132,182–187]. This may help to explain why cluster C also had higher levels of ACE soil protein, a measure of potentially available organic nitrogen[76,77], than the other two clusters. Taken together, the long-term use of animal integration to manage and convert residues in the orchards from cluster C provide evidence that ICL systems might be an effective strategy to supply soil fertility in this particular agroecosystem.

In addition to nitrogen, cluster C also had significantly higher levels of plant available P. While only significantly different across both clusters in the Tree, cluster C had approximately 13 times more available P than cluster A and 2.9 (Alley) and 3.3 (Tree) times more available P than cluster B. This finding is notable because P is highly immobile in the soil and often exists in organic forms that are unavailable to plants[188]. It is also consistent with a broader body of work that shows the benefit of moderate animal grazing for increasing plant available P in the soil[49,188,189]. Similar to that of other nutrients, animals return dung and urine to the soil after grazing events that have high concentrations of bioavailable P[179,190]. Animal integration may thus be particularly useful for P management in perennial agroecosystems – like the ones from this study – because interrow vegetation that has accumulated soil P could be processed by animals and subsequently returned to the soil. Although animal integration has demonstrated a potential to improve supplies of certain soil nutrients, available K was not significantly different across the three orchard clusters. Given that nut crops have a particularly high K demand[191],

identifying strategies to maintain sufficient K supply overtime is of critical importance to the long-term sustainability of these agroecosystems[192].

4.5 Inherent factors as a major determinant of soil physical properties

In contrast to the other indicators measured in this study, the indicators selected to represent the soil's capacity to improve soil structure and supply water did not demonstrate many meaningful differences across the three orchard clusters. Soil compaction, as measured by bulk density, was 9% lower in the Alley zone of cluster C compared to cluster A, while cluster B was intermediate and not significantly different. Although these differences did result in decreased soil bulk density, the differences in compaction as it influences root growth restriction is marginal. The mean bulk densities across all three clusters in the Alley and Tree zone had values that were categorized as potentially restrictive to root growth for the relevant soil textural category[193–195]. Soil compaction in the heavy clay-dominant soils characteristic of this study region[50] may be further exacerbated by the use of no-tillage, which has been shown to increase bulk density, restrict root growth, and slow N mineralization in similar agroecosystems[60]. The influence of inherent soil properties may also partially explain the similarities of additional measured indicators like wet aggregate stability and available water-holding capacity across the three orchards. Multiple meta-analysis indicate that soil texture, and particularly clay content, has a outsized influence of the impacts of popular soil health building strategies like living covers[43,44], diversification[38,196], animal grazing[44,189], and residue retention[197] on physical indicators related to soil structure and water. Specifically, soils with higher clay contents may not show as pronounced improvements with the implementation of these management practices when compared to coarser soil textures[43,44,189]. This may not be

surprising as the inherent textural components of a soil have a considerable impact its physical characteristics[198], such as wet aggregate stability and available water content[199]. This result was, however, unexpected as other soil properties thought to be highly influential in enhancing soil water storage – such as soil organic carbon[198,200] – differed between clusters but didn't translate to meaningful differences in measured physical characteristics. It may therefore be useful and necessary to further explore the impacts of soil health building strategies on indicators of soil structure and water dynamics in the irrigated lands of this agroecosystem– as well as investigate the capacity to realize substantial improvements in water retention and cycling in these semi-arid agroecosystems.

5. Conclusion

The findings from this study demonstrate the there is potential to rebuild healthy soils and enhance soil ecosystem functionality in California almond agroecosystems. Orchards that utilized animal integration reflected a diverse and stacked application of soil health building principles and had the most multifunctional soils. Specifically, indicators of the soils capacity to build soil carbon, supply fertility, and support diverse and active soil communities were highest in these orchards. While these findings add to the growing body of work demonstrating the potential that ICL systems could unlock a more multifunctional agroecosystem, farmers across California remain skeptical over the feasibility of animal integration given strict food safety compliance standards. It may therefore be necessary to pair more research onto the soil ecosystem functional benefits of these systems with an exploration of the potential food safety risks. In contrast to research from similar agroecosystems, orchards that applied organic amendments with or without cover crops clustered with bare soil management (cluster A) and

consistently had the lowest values for measured indicators across the soil ecosystem functions. Given that these practices are increasingly being adopted across much of California – and are frequently promoted for their purported soil health benefits – we urgently need more research to validate the capacity for these specific practices to meaningfully improve soil ecosystem functionality. The physical soil properties that served as indicators of the soil's capacity to improve soil structure and supply water did not meaningfully differ across clusters in this study. Further work exploring to what extent soil health building practices can improve the physical characteristics that influence soil structure and water cycling is of particular importance because much of the agroecosystems across California are irrigated and located in semi-arid climates - that are also increasingly undergoing aridification under climate change.

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Figures

Figure 1: (A) Each orchard was detailed in terms of how soil management translated to the five soil health building principles in both the center Alley and Tree berm management zones. **(B-D)** Orchards selected for this study ranged in management from bare soils **(B)** and variety of living covers to extensively grazed vegetative understories **(C-D)**. **(E)** Orchard soil management practices documented for the alley and tree berm reflected multiple individual and stacked applications of soil health building principles. Abbreviations were defined as follows: S (synthetic inputs), O (organic inputs), NT (no-till), AM (animal manure), LC (plant living cover), OA (organic amendments). Blank cells indicate that the principle was not implemented. Additional management information is given about living cover termination method and alternative irrigation systems if they were not micro-irrigation systems.
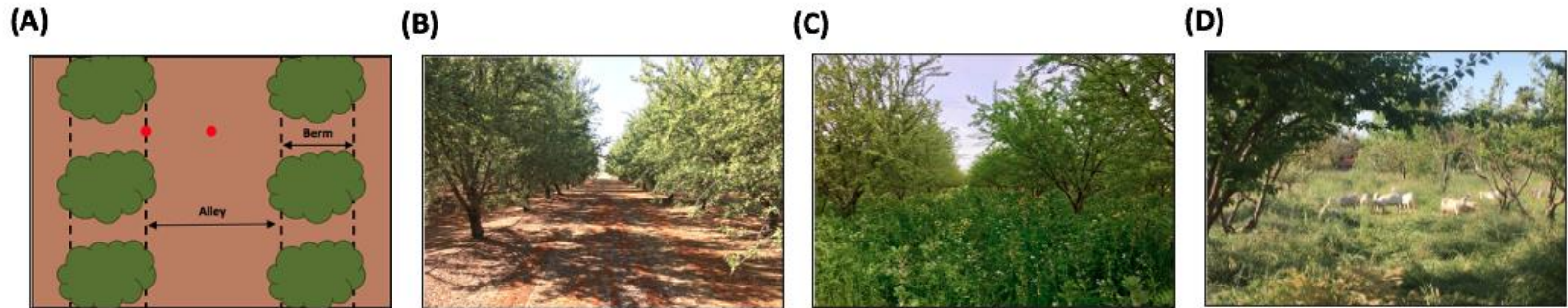
Site	Soil type	Farm input	Physical disturbance Tree berm	Alley	Plant living covers Tree berm	Alley	Soil cover Tree berm	Alley	Input of organic matter Tree berm	Alley	Diversification strategies Tree berm	Alley	Additional management
1	Silty clay	S	NT	NT			Bare	Bare					
2	Silty clay loam	S	NT	NT			Bare	Bare					
3	Clay loam	O	NT; Animal	NT; Animal	Continuous	Continuous	Full	Full	AM; LC	AM; LC	Forage mix; cattle, sheep, chicken	Forage mix; cattle, sheep, chicken	Overhead sprinklers
4	Silty clay loam	S	NT	NT		Winter	Bare	Partial		OA; LC		Grass cover crop	Mow terminated
5	Clay loam	S	NT	NT		Winter	Bare	Partial		LC		Resident veg	Herbicide terminated
6	Silty clay loam	S	NT	NT			Bare	Bare					
7	Silty clay loam	O	NT; Animal	NT; Animal	Continuous	Continuous	Full	Full	AM; LC	AM; LC	Chicken; resident veg	Chicken; resident veg	Overhead sprinklers
8	Loam	S	NT	NT			Bare	Bare	OA	OA			
9	Loam	O	NT; Animal	NT; Animal	Winter	Winter	Partial	Partial	OA; AM; LC	OA; AM; LC	3 species cover crop; sheep	3 species cover crop; sheep	Graze terminated; overhead sprinklers
10	Loam	S	NT	NT		Winter	Bare	Partial		LC		3 species cover crop	Mow terminated
11	Loam	S	NT	NT		Continuous	Bare	Full		LC		3 species cover crop /resident veg	Mowed preceding harvest
12	Silt loam	S	NT	NT		Winter	Bare	Partial		LC		Resident veg	Herbicide terminated

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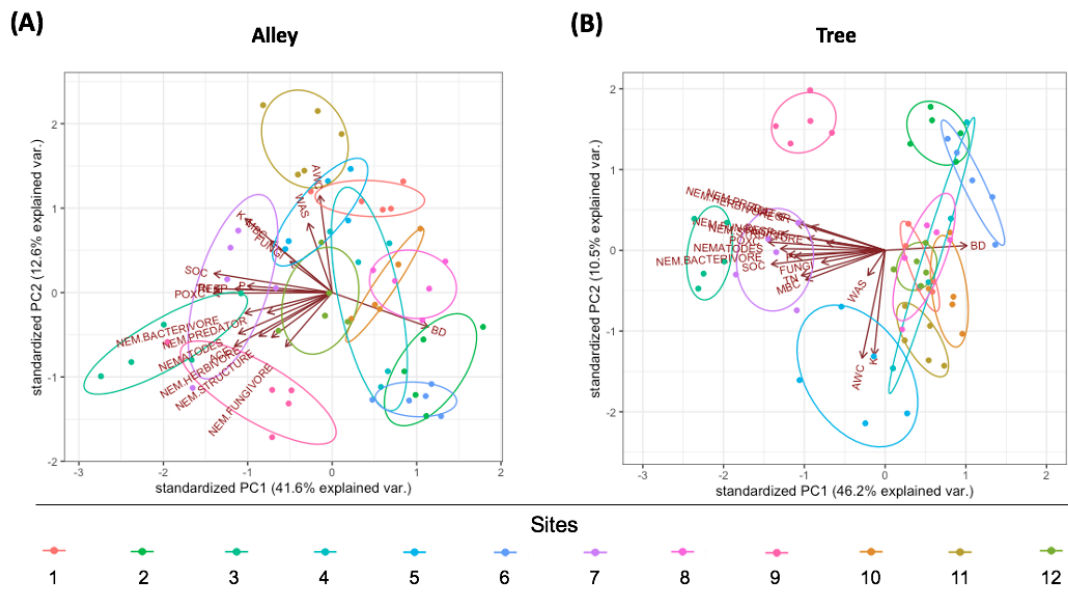
Figure 2: Principal component analysis was generated for the Alley **(A)** and Tree **(B)** data to explore relationships between the 12 orchards and measured indicators of soil ecosystem functions. Samples are represented as dots and color coded by site. The vectors represent measured indicators of soil ecosystems as outlined in **Table 1**. A k-means cluster analysis for the Alley **(C)** and Tree **(D)**, where the large dots represent the centroid of each cluster, and the small dots represent samples.
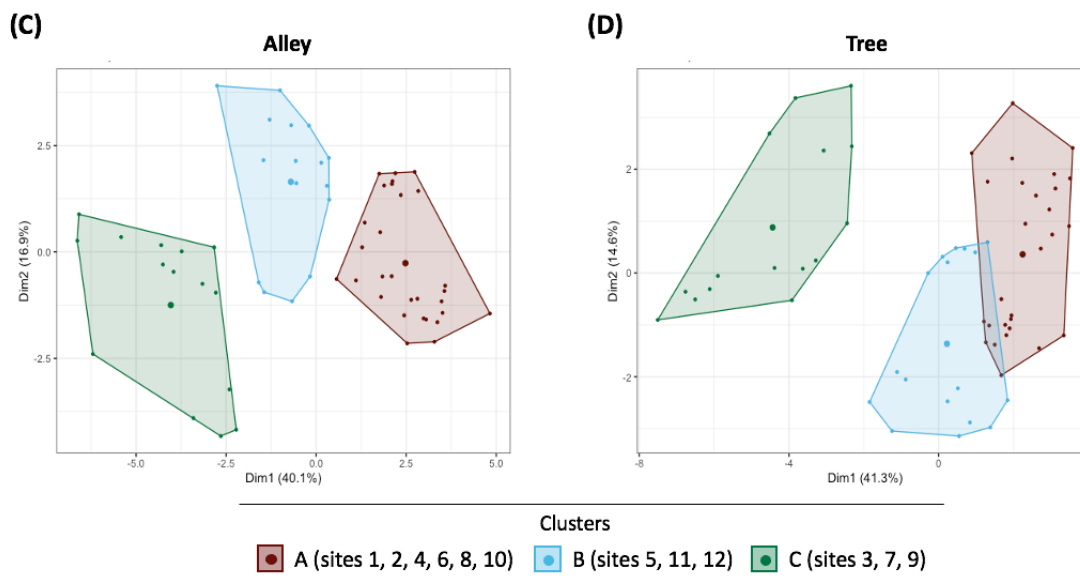
Figure 3 : Normalized values of the selected soil ecosystem functions by cluster for the **(A)** Alley and **(B)** Tree zones. An index was calculated for each cluster to evaluate overall soil ecosystem multifunctionality (MF) in the **(C)** Alley and **(D)** Tree zones. Abbreviations for the indicators can be found in **Table 1**.
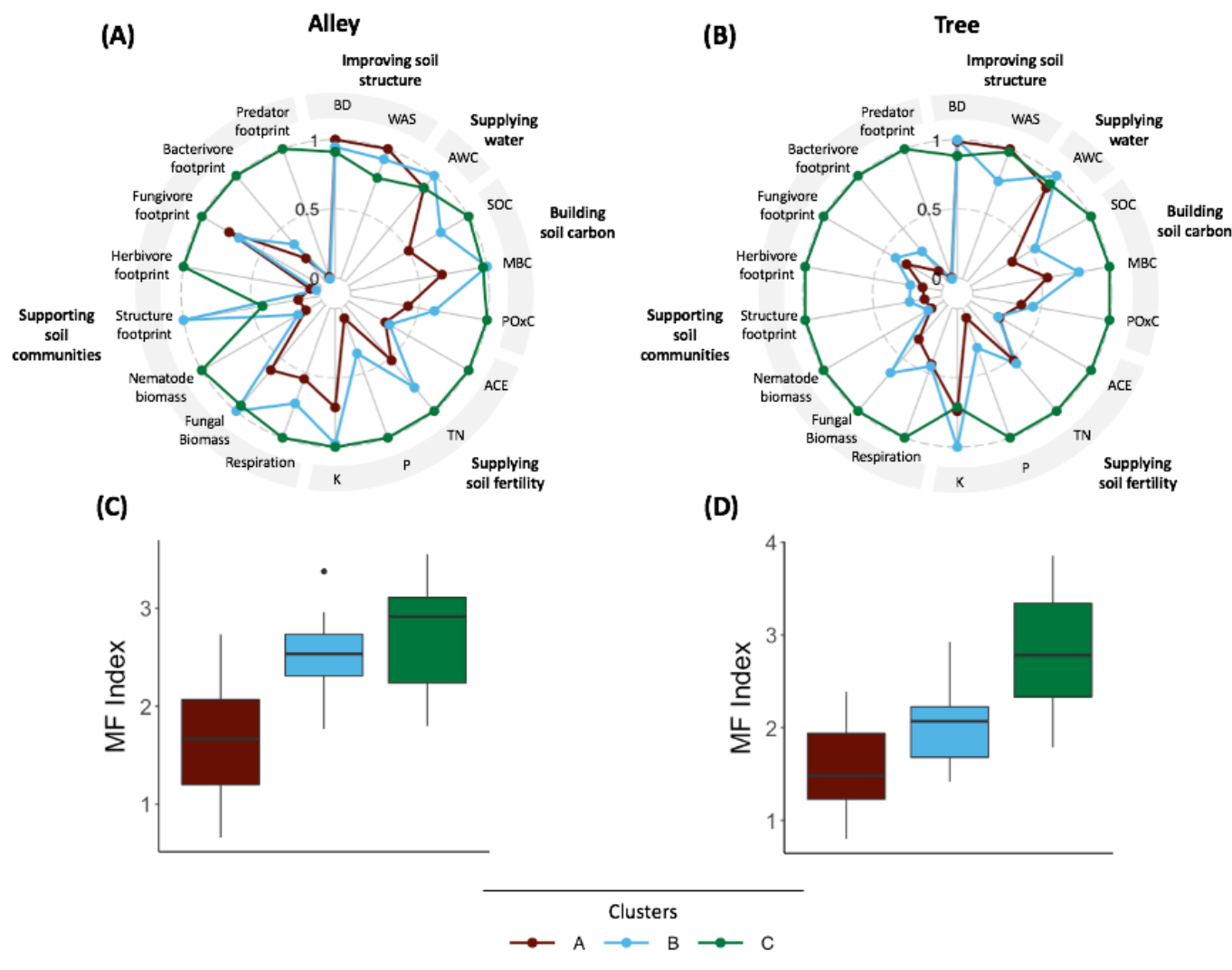
Table 1: Soil Ecosystem functions and their respective biological, physical, and chemical indicators. Abbreviations used throughout text are designated in parentheses next to each indicator.

Soil ecosystem function	Indicators	Abbreviation
Improving soil structure	Bulk density	BD
	Wet aggregate stability	WAS
Supplying soil water	Available water-holding capacity	AWC
Building soil carbon	Soil organic carbon	SOC
	Microbial biomass carbon	MBC
	Permanganate oxidizable carbon	POxC
Supplying soil fertility	Autoclaved-citrate extractable soil protein	ACE
	Total Nitrogen	TN
	Available phosphorus	P
	Available potassium	K
Supporting diverse and active soil communities	Respiration	RESP
	Fungal biomass	FUNGI
	Nematode biomass	NEMATODES
	Structure footprint	NEM.STRUCTURE
	Herbivore footprint	NEM.HERBIVORE
	Fungivore footprint	NEM.FUNGIVORE
	Bacterivore footprint	NEM.BACTERIVORE
	Predator footprint	NEM.PREDATOR

Table 2: Orchard cluster means and standard errors for the select indicators of soil ecosystem functions in the 0-20 cm depth zone for the Alley and Tree. Significant differences at a level of P < 0.05 are indicated by capital letters, and trends at a level of P <0.1 are indicated by lowercase letters.

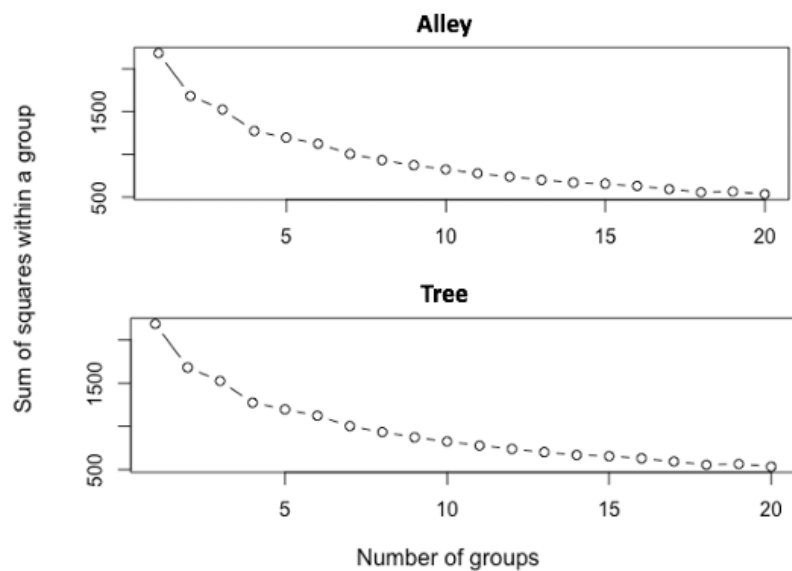| Soil ecosystem functions	Indicators	Units	Alley Cluster A		Alley Cluster B		Alley Cluster C		Tree Cluster A		Tree Cluster B		Tree Cluster C	
Improving soil structure	Bulk density	g/cm^3	1.71 ± 0.02	A	1.62 ± 0.02	AB	1.56 ± 0.03	B	1.61 ± 0.02	a	1.63 ± 0.21	ab	1.44 ± 0.04	b
	Wet aggregate stability	%	26.27 ± 3.01		24.17 ± 4.32		20.42 ± 2.41		27.15 ± 3.18		20.43 ± 3.62		26.56 ± 3.27	
Supplying soil water	Available water-holding capacity	g water/ g soil	0.23 ± 0.004		0.26 ± 0.005		0.23 ± 0.006		0.23 ± 0.004		0.26 ± 0.006		0.24 ± 0.005	
Building soil carbon	Soil organic carbon	g C/ kg dry soil	8.78 ± 0.41	A	13.43 ± 0.47	B	17.47 ± 1.03	B	7.83 ± 0.22	A	12.17 ± 0.59	B	22.64 ± 2.04	C
	Microbial biomass carbon	ug C/g soil	254.11 ± 21.77		378.70 ± 27.84		367.70 ± 24.49		204.00 ± 16.95	A	289.50 ± 21.65	AB	372.20 ± 24.49	B
	POxC	ppm	277.70 ± 8.50	A	404.20 ± 9.42	B	656.00 ± 34.60	C	265.30 ± 6.37	A	328.38 ± 11.71	A	742.41 ± 54.03	B
Supplying soil fertility	ACE soil protein	mg N/g dry soil	1.81 ± 0.05	A	2.01 ± 0.13	A	5.89 ± 0.45	B	1.90 ± 0.06	A	1.81 ± 0.16	A	7.93 ± 0.69	B
	Total nitrogen	g N/kg dry soil	0.85 ± 0.03	A	1.27 ± 0.05	B	1.63 ± 0.09	C	1.09 ± 0.31	A	1.15 ± 0.07	A	2.09 ± 0.19	B
	Available phosphorus	ppm	4.64 ± 0.51	A	20.12 ± 2.67	B	57.59 ± 11.47	B	4.70 ± 0.62	A	18.73 ± 2.63	B	61.24 ± 7.94	C
	Available potassium	ppm	206.82 ± 10.29		282.90 ± 12.38		289.47 ± 17.39		281.01 ± 18.81		379.00 ± 32.37		269.35 ± 26.80	
Supporting diverse and active soil communities	Respiration	mg c02/g dry soil	0.29 ± 0.01	A	0.39 ± 0.04	AB	0.53 ± 0.04	B	0.30 ± 0.01	A	0.31 ± 0.02	A	0.69 ± 0.06	B
	Fungal biomass	nmol/g soil	163.74 ± 34.05		267.56 ± 54.96		253.48 ± 63.06		107.20 ± 19.58	a	213.18 ± 34.83	ab	333.90 ± 68.83	b
	Nematode biomass	mg/ g soil	1.26 ± 0.18	A	1.87 ± 0.39	AB	9.41 ± 5.13	B	1.41 ± 0.35	A	3.56 ± 1.78	A	13.46 ± 3.22	B
	Structure footprint	µ C /200 ml	13.29 ± 4.87	a	82.06 ± 71.10	ab	35.00 ± 13.84	b	11.09 ± 2.71	a	20.34 ± 8.04	ab	85.46 ± 31.44	b
	Herbivore footprint	µ C /200 ml	65.69 ± 22.27	A	25.28 ± 6.61	A	902.51 ± 740.57	B	58.04 ± 18.06	A	93.93 ± 73.32	A	404.80 ± 92.44	B
	Fungivore footprint	µ C /200 ml	47.52 ± 8.21		42.47 ± 11.69		61.43 ± 10.04		35.71 ± 5.86	A	46.18 ± 16.49	A	114.73 ± 28.37	B
	Bacterivore footprint	µ C /200 ml	251.84 ± 41.91	A	403.45 ± 82.09	AB	1148.50 ± 327.18	B	311.51 ± 95.03	A	885.78 ± 438.94	AB	3113.30 ± 787.95	B
	Predator footprint	µ C /200 ml	0.20 ± 0.20	A	0.00 ± 0.00	A	14.10 ± 6.77	B	0.58 ± 0.32	A	0.00 ± 0.00	A	55.70 ± 25.85	B

Supplementary figures and tables

Figure S1: A k-means cluster analysis was used to identify underlying categorical structure in the alley and tree datasets. **(A)** Scree plots were used as an initial tool to identify potential k cluster options. **(B)** Gap statistics were one of multiple statistical validation tools used to select the final k value for orchard clusters for the alley and tree.
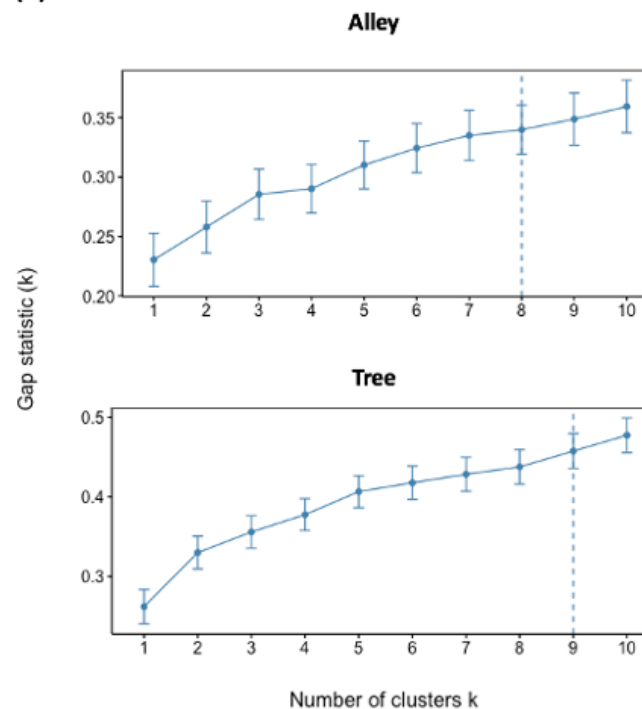
Table S1: Means and standard errors were calculated for the select indicators of soil ecosystem functions by site in the 0-20 cm depth zone for the alley and tree. Abbreviation for the soil ecosystem function indicators can be found in Table 1.

Alley

Soil ecosystem functions	Indicators	Units	1 Mean	1 SE	2 Mean	2 SE	3 Mean	3 SE	4 Mean	4 SE	5 Mean	5 SE	6 Mean	6 SE	7 Mean	7 SE	8 Mean	8 SE	9 Mean	9 SE	10 Mean	10 SE	11 Mean	11 SE	12 Mean	12 SE
Improving soil structure	Bulk density	g/cm^3	1.61	0.02	1.79	0.04	1.42	0.02	1.67	0.05	1.65	0.02	1.76	0.03	1.67	0.03	1.71	0.01	1.58	0.04	1.73	0.02	1.53	0.03	1.67	0.02
	WAS	%	49.66	0.80	11.77	1.08	30.99	1.89	40.55	5.59	14.65	1.41	14.10	1.28	19.27	2.25	10.82	0.63	10.99	1.41	30.70	4.00	46.33	2.69	11.52	1.24
Supplying soil water	AWC	g water/ g soil	0.24	0.002	0.20	0.003	0.21	0.003	0.22	0.013	0.28	0.002	0.23	0.004	0.25	0.002	0.23	0.006	0.21	0.008	0.25	0.003	0.27	0.004	0.24	0.004
Building soil carbon	SOC	g C/ kg dry soil	11.24	1.40	7.40	0.45	20.19	2.06	10.83	0.65	13.95	0.77	7.79	0.48	16.05	0.79	7.57	0.61	16.17	1.88	7.83	0.53	13.43	0.85	12.90	0.94
	MBC	ug C/g soil	294.68	45.70	63.23	14.90	341.33	47.53	318.99	43.41	352.63	47.21	205.24	25.55	419.81	41.73	351.11	33.09	341.91	35.76	291.43	28.46	362.44	59.98	421.12	40.33
	POxC	ppm	346.58	14.64	267.25	19.16	789.64	29.42	247.87	12.00	413.53	4.26	274.96	5.75	600.16	14.45	242.51	18.94	578.29	68.87	287.30	15.95	399.82	17.61	399.37	23.89
Supplying soil fertility	ACE	mg N/g dry soil	1.68	0.04	1.75	0.14	5.47	0.42	1.92	0.11	2.12	0.09	2.11	0.06	5.01	0.19	1.53	0.07	7.18	1.14	1.85	0.17	1.47	0.08	2.44	0.21
	Total N	g N/kg dry soil	0.87	0.13	0.73	0.03	1.83	0.20	1.06	0.06	1.27	0.07	0.84	0.04	1.58	0.07	0.76	0.05	1.48	0.16	0.87	0.05	1.24	0.07	1.29	0.11
	Available P	ppm	2.95	0.09	9.16	1.36	15.23	1.15	4.26	0.98	19.49	2.26	3.08	0.42	89.83	15.87	5.46	0.87	67.72	19.85	2.94	0.36	30.41	4.21	10.44	1.49
	Avialable K	ppm	288.11	12.68	186.31	10.46	334.38	11.53	232.95	14.99	313.01	8.16	118.14	2.74	329.52	14.19	199.34	11.08	204.73	11.81	215.90	7.18	308.69	14.40	227.03	12.70
Supporting diverse and active soil communities	Respiration	mg c02/g soil	0.28	0.02	0.28	0.02	0.68	0.08	0.33	0.05	0.25	0.01	0.24	0.02	0.43	0.06	0.31	0.04	0.49	0.04	0.34	0.03	0.58	0.02	0.35	0.02
	Fungal biomass	nmol/g soil	374.85	116.65	192.49	61.56	438.23	140.75	22.79	8.18	174.36	66.05	32.50	11.09	122.30	55.47	249.09	95.46	199.89	72.84	110.71	24.07	227.76	66.01	400.57	129.50
	Nematode biomass	mg/ g soil	0.62	0.14	0.83	0.23	23.15	14.26	1.89	0.54	2.12	0.08	1.45	0.72	3.22	1.41	0.97	0.26	1.87	0.43	1.81	0.34	1.00	0.27	2.49	0.69
	Structure footprint	C units/kg soil	3.00	1.28	4.15	2.40	19.50	6.27	4.48	3.93	17.37	13.14	53.19	19.76	58.31	41.29	0.34	0.34	27.20	7.49	14.59	10.50	7.45	2.79	221.35	213.55
	Herbivore footprint	C units/kg soil	48.93	28.24	61.29	33.23	2499.0	2191.1	229.90	103.92	23.14	8.69	32.96	13.61	90.46	35.09	9.40	6.26	118.03	23.05	11.65	5.98	29.79	15.20	22.91	12.01
	Fungivore footprint	C units/kg soil	29.05	9.30	16.66	3.53	92.40	19.79	39.34	8.61	38.53	13.60	66.37	20.22	37.09	12.41	40.68	11.94	54.79	10.67	93.04	35.31	17.75	8.07	71.12	28.70
	Bacterivore footprint	C units/kg soil	111.24	37.31	142.05	26.52	2280.1	699.61	206.36	51.29	555.37	205.19	316.18	203.93	775.53	347.71	292.65	71.36	389.95	122.00	441.56	77.72	251.45	72.01	403.52	109.17
	Predator footprint	C units/kg soil	1.20	1.20	0.00	0.00	9.45	6.87	0.00	0.00	0.00	0.00	0.00	0.00	30.02	18.02	0.00	0.00	2.83	2.38	0.00	0.00	0.00	0.00	0.00	0.00

Tree

Soil ecosystem functions	Indicators	Units	1 Mean	1 SE	2 Mean	2 SE	3 Mean	3 SE	4 Mean	4 SE	5 Mean	5 SE	6 Mean	6 SE	7 Mean	7 SE	8 Mean	8 SE	9 Mean	9 SE	10 Mean	10 SE	11 Mean	11 SE	12 Mean	12 SE
Improving soil structure	Bulk density	g/cm^3	1.45	0.04	1.69	0.05	1.26	0.03	1.56	0.03	1.59	0.03	1.68	0.02	1.57	0.02	1.63	0.02	1.49	0.04	1.67	0.05	1.58	0.02	1.71	0.01
	WAS	%	53.58	2.11	13.58	1.22	40.78	4.89	43.23	5.47	12.85	0.68	17.99	1.61	22.36	1.83	8.28	0.57	16.54	2.22	26.27	2.15	39.03	2.27	9.42	0.76
Supplying soil water	AWC	g water/ g soil	0.24	0.001	0.20	0.001	0.24	0.004	0.23	0.015	0.28	0.001	0.23	0.003	0.26	0.003	0.24	0.003	0.21	0.002	0.25	0.003	0.26	0.002	0.24	0.005
Building soil carbon	SOC	g C/ kg dry soil	8.91	0.83	7.72	0.15	32.14	2.42	8.68	0.50	14.63	0.69	7.13	0.22	18.47	1.31	7.37	0.47	17.30	1.44	7.19	0.31	10.73	0.27	11.14	0.88
	MBC	ug C/g soil	221.61	21.80	109.01	24.56	376.55	25.89	248.93	42.91	297.42	38.48	175.12	18.63	409.31	44.79	306.09	48.89	330.71	53.33	163.21	27.86	255.87	47.69	315.08	26.57
	POxC	ppm	306.02	11.86	271.84	15.69	1012.4	45.81	228.83	9.45	381.11	13.68	261.20	12.06	634.02	13.06	272.49	13.00	580.76	26.27	251.68	12.28	288.01	6.95	316.13	7.99
Supplying soil fertility	ACE	mg N/g dry soil	1.78	0.12	2.11	0.18	11.01	1.01	1.96	0.17	2.36	0.11	2.07	0.19	5.99	0.41	1.77	0.15	6.79	0.46	1.71	0.09	1.03	0.05	2.04	0.08
	Total N	g N/kg dry soil	0.75	0.11	0.71	0.01	2.98	0.21	2.75	1.84	1.35	0.10	0.80	0.03	1.74	0.10	0.71	0.05	1.55	0.16	0.81	0.02	1.02	0.04	1.08	0.15
	Available P	ppm	2.15	0.37	4.89	0.52	34.84	3.88	3.78	0.56	20.03	1.32	3.89	1.32	81.59	11.17	7.97	2.99	67.30	15.33	5.48	0.82	27.50	5.05	8.65	0.62
	Avialable K	ppm	289.61	14.60	203.52	12.11	349.72	9.97	319.15	35.85	466.08	64.06	466.02	46.10	161.62	19.53	323.20	42.24	135.13	14.80	367.74	38.95	360.62	35.94	310.92	49.67
Supporting diverse and active soil communities	Respiration	mg c02/g soil	0.35	0.04	0.33	0.03	1.00	0.05	0.24	0.01	0.28	0.02	0.25	0.03	0.53	0.03	0.35	0.03	0.53	0.01	0.28	0.02	0.26	0.04	0.28	0.03
	Fungal biomass	nmol/g soil	205.03	54.20	146.16	43.38	535.63	150.46	77.68	40.40	271.65	79.87	17.35	3.19	153.38	61.65	118.07	60.60	312.69	69.75	78.90	36.12	190.24	38.69	177.64	59.68
	Nematode biomass	mg/ g soil	0.95	0.59	1.87	0.86	17.27	4.58	0.90	0.24	8.51	4.89	0.45	0.19	11.14	3.27	3.52	1.54	11.97	8.46	0.80	0.31	0.98	0.50	1.30	0.53
	Structure footprint	C units/kg soil	11.26	5.91	11.90	4.48	37.27	11.29	4.17	3.48	51.89	16.82	23.90	10.60	190.08	76.08	2.42	2.10	29.04	18.69	12.89	7.97	2.42	0.87	6.72	4.50
	Herbivore footprint	C units/kg soil	103.77	89.21	54.16	28.19	430.82	159.08	152.09	31.64	242.11	218.76	12.34	9.61	207.36	37.41	13.63	5.99	576.30	213.87	12.26	4.79	13.27	8.61	26.40	16.22
	Fungivore footprint	C units/kg soil	42.22	12.42	19.95	4.48	185.07	72.45	16.85	3.64	81.25	45.06	43.63	28.15	75.48	9.92	53.65	13.07	83.65	35.17	38.15	9.27	16.62	7.58	40.66	15.28
	Bacterivore footprint	C units/kg soil	89.12	13.29	495.04	259.82	4182.2	1185.2	89.38	33.12	2096.7	1200.9	70.69	39.12	2650.9	797.78	947.88	402.78	2506.8	2011.8	176.92	82.56	252.60	128.00	308.00	118.96
	Predator footprint	C units/kg soil	1.07	1.07	2.39	1.47	19.41	9.81	0.00	0.00	0.00	0.00	0.00	0.00	142.36	63.76	0.00	0.00	5.33	3.77	0.00	0.00	0.00	0.00	0.00	0.00