Integrating Cover Crops and Almond Hulls and Shells as Organic Matter Amendments for Whole Orchard Regenerative Management

By

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Abstract

Regenerative agricultural practices strive to produce food that have lower economic, environmental, and social impacts defined by the processes used (i.e. using cover crops, reducing tillage) or by proposed outcomes (i.e. improving soil health, improving biodiversity). In almond orchards the regeneration of ecosystem health and productivity can be achieved through practices including recycling of almond hull and shell resources as soil amendments, reducing dust production during harvest, and minimizing synthetic fertilizer inputs. The current and set standards of conventional almond production poses a unique challenge when proposing regenerative agriculture goals. To facilitate almond harvest and simplify irrigation and fertigation practices, almond orchards are typically managed with the orchard floor bare, and trees are grown in rows with irrigation and fertilization localized exclusively to the 3.6 meter (12-ft) wide soil strip below the tree canopy. The alleyways are often left unirrigated and bare to facilitate almond harvest. These conventional practices reduce soil health, whereas with the advent of off-ground harvesting, opportunities exist to incorporate cover crops (CC) and the use of organic matter amendments (OMA) including almond hulls and shells (AHS) to maintain a more permanent mulch layer to protect the soil. This thesis examined the changes in soil physical, chemical, and biological properties from the use of AHS and a CC mix of 60% oats, 35% spring peas, and 5% yellow mustard in a randomized complete block design field trial. Nutrient cycling and OMA decomposition dynamics was analyzed one and two years after practice adoption. Effects on soil aggregate stability, particulate organic matter (POM), mineral associated organic matter (MAOM), microbial biomass, and soil moisture in amended trees harvested using on-ground and off-ground harvest machines were determined. Results showed increased C and N in microaggregate and macroaggregate soil organic matter fractions, increased saprophytes and arbuscular mycorrhizal fungi, and improved soil moisture in amended soils. Exchangeable potassium (XK) and soil organic matter (SOM) increased in the amended on and off-ground harvest treatments and was highly correlated with microbial communities measured. We found that after 3 years of AHS OMA and catch-frame harvest, MAOM and soil aggregate stability increased due to this undisturbed forest litter layer which may allow more microbial processes to contribute to more MAOM over time. Cover crop biomass was highest in oats but peas contributed the highest soil N. 192 days after AHS application, an average of 38% of the AHS material decomposed and 90 days after the CC were seeded, an average of 69% of the CC material decomposed.

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Chapter 1: Management of organic matter amendments and cover crops in orchards impacts soil ecosystems

1.1 Introduction

The farming systems of regenerative, conservation, and sustainable agriculture have similar principles to improve soil health using practices such as organic matter amendments (OMA), mulching, growing cover crops and green manures, composting, and crop rotation with the intention of building soil organic matter (SOM) (Newton et al., 2020; Rhodes, 2017). While examples of the benefits of each practice separately are available, information on the combined effects of cover crops (CC) and organic matter amendments (OMA) in orchard systems is scarce.

The three general categories of cover crops by species include grasses (oats, rye, millet), broadleaves (flax, mustard, safflower), and legumes (peas, clovers, vetch). Each species has unique properties that when utilized properly, can alleviate potential issues an orchard faces. Grass species can reduce bulk density, legume species can build soil nitrogen (N) over time, and broadleaf species decompose quickly to aid in nutrient release into the soil (Koudahe et al., 2022). Understanding the specific issues in an orchard such as reducing nutrient tie up from high carbon inputs, will allow growers to determine the best mix of legumes and grasses to combat this issue. Utilizing multiple species CC mixes is an important tool to managing soil health concerns and has been commonly used in Mediterranean climates to prevent soil erosion loss during summer droughts and to facilitate machinery driving in wet conditions during fall rain (Dupraz et al., 2018). A subset of OMA byproducts includes nutshells, straw, hazelnut husks, and other plant material typically used as waste. Intensive agriculture has depleted much of the SOC stocks, which creates an opportunity for OMA to aid in the restoration of previously fertile land. Applying a mulch layer of OMA in orchard agroecosystems (Andrews et al., 2021) has the potential to improve soil organic carbon (SOC), soil structure, and improve water availability (Andrews et al., 2021; Hodson et al., 2021; Ory et al., 2022). This review will synthesize current research on applied organic matter amendments and cover crops in orchard systems to better understand their implications on soil health practices relevant to our research goals to evaluate aggregate stability, SOM fractions, microbial communities, and regenerative management practices in California almond orchards.

1.2 Importance of organic matter amendments and cover crops on soil health

1.2.1 Organic matter amendments

OMA sources include crop residues, compost, biochar, mulch, and leaf litter. These classes of OMA can be used as supplemental macronutrients for crops that include nitrogen (N), phosphorus (P), and potassium (K). In addition to these mineral source benefits, maintaining soils with amendments would encourage soil microorganisms to feed on decomposing plant litter to form soil organic matter (SOM) which is commonly studied in temperate forests, where leaf litter OMA naturally builds SOM over time (Currie et al., 1996; Dupraz et al., 2018; Sayer et al., 2021; C. Wang et al., 2018). Fungal taxa are particularly susceptible to high N inputs from leaf litter decomposition resulting in lower fungi to bacteria ratios and can depolymerize organic matter by producing extracellular enzymes (Wang et al., 2018; Wei et al., 2022). In an experiment studying the effects from root litter and leaf litter inputs in forest systems, phospholipid fatty acid (PLFA) analyses and microbial biomass were evaluated to group microbes by species (Joergensen, 2022; Wei et al., 2022). Researchers discovered that litter additions significantly increased the bacterial, fungal, and gram-negative bacterial PLFAs, but had no effect on gram-positive bacteria. This suggested labile C preference from microbes based on the overall weighted percentage changes in the soil from the addition of litter inputs (Song et al., 2020; Wu et al., 2017; Yang & Chen, 2009; Zhang et al., 2020).

SOM not only contains soil organic carbon (SOC) useful for C sequestration, but it also contains other important macronutrients including N, P, K, sulfur (S), and calcium (Ca) (Dynarski et al., 2020). SOM serves as a vital substrate for microbial metabolism and for long term storage of C that can improve soil health which is defined as, "…the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (USDA NRCS, 2023). An alternative definition of soil health has been more recently described as a hierarchical framework that aims to create individual and integrated categories for (1) signs of life, (2) signs of function, (3) signs of complexity, and (4) signs of emergence (Harris et al., 2022). Harris et al., 2022 proposed that soil health should be assessed via a whole systems approach through characterizing (1) communities that exist in the soil (DNA, organic carbon profiling), (2) soil ecosystems and how materials respond to different abiotic and biotic inputs (catabolic profiling, thermodynamic efficiency), (3) isolated individuals and populations that are active across different scales (community trophic structures, interactions between soils and plants), and (4) soil systems exposed to multiple stressors (repeated perturbation, recovery response). The researchers argued that soil is not a living organism and cannot be measured quantifiably, so to assess for soil health, using larger time scales to identify the components, complexity, and function of soil can produce a more complete assessment. These definitions demonstrate nuanced approaches to defining soil health and our study will focus on community and nutrient responses from applied crop residues.

Examples of OMA that build SOM include rice husks, nutshells, and compost. One such OMA that has been proposed as an amendment to increase SOC are rice husks which aid in the suppression of methane (CH4) emissions that rice grains may exacerbate (Linam et al., 2023). Their results suggested that husk amendments store more C as SOC than they emit as CH4 or as carbon dioxide (CO2), contributing to future research utilizing crop waste products as alternative

C sources. Nutshell amendments typically have a higher C concentration, and when applied to the soil, may shift microbial communities to fungi dominated soils which are most capable of utilizing C substrates (Andrews et al., 2021; Jackson et al., 2017). With increased C inputs belowground, interactions between roots and microbes can lead to priming effects and aggregate formation that would improve SOM formation with continued nutshell OMA applications. Compost is one other amendment that has been shown to moderate soil pH, increase organic matter, and increase nutrient concentrations (Andrews & Kassama, 2022; Larkin, 2020; Wong et al., 2023). Dairy manure and green waste compost may reduce soil bulk density and compaction, prevent N leaching, and improve water use efficiency which is a prevalent issue in almond orchards in California (Khalsa et al., 2022; Lepsch et al., 2019). To combat soil erosion, OMA have been suggested to improve soil stability by protecting aggregate surfaces against rainfall impact and providing substrates for microbes to adhere to as stable soil particles. One measurement to assess for soil stability is to calculate the proportion of the dry mass of soil relative to its bulk volume in units of g/cm^3, otherwise known as soil bulk density. More compacted soils have higher bulk density values, with most trees preferring well drained soils with smaller bulk density values (Rivenshield & Bassuk, 2007; Zebarth et al., 1999). Experiments have shown that soil bulk density decreased with almond shell, hazelnut husk, and alfalfa mulch amendments, and improved soil aggregate stability which has been linked to high microbial activity and improved soil structure (Franzluebbers, 2022; Kremer & Kussman, 2011).

1.2.2 Cover crops

Cover crops (CC) have important significance on soil physical, chemical, and biological properties, and when combined with other amendments, these stacked practices can contribute to a more diverse soil microbiome. Soil physical benefits from CC include improvements in wet and dry soil aggregate stability, bulk density, and porosity. Soil chemical benefits may increase SOM, nutrients such as N and P, cation exchange capacity (CEC), and electrical conductivity (EC). Biological soil benefits may result in an abundance of nematodes, earthworms, fungi, bacteria, and other microbes that may be strongly associated with certain CC species (Hodson et al., 2021; Koudahe et al., 2022; Martínez-Mena et al., 2021).

Cover crop species can be seeded as a combination of grasses (oats, rye, millet), legumes (peas, clovers, vetch), and non-leguminous broadleaves (buckwheat, mustard-brassica, safflower) or as a singular species depending on the specific goals and environmental conditions in each agricultural landscape. Grass species are beneficial due to their ability to provide a substantial soil cover to reduce bulk density and increase water stability (Koudahe et al., 2022). Legume species are utilized for their ability to fix atmospheric N that can be built up over time to supply main crops. Non-legume broadleaf species can rapidly decompose faster than grass species and release vital nutrients to the soil as they decay. Brassicas, such as mustard, also have specific tap roots that provide aeration and deeper rooting systems that may improve soil structure (Alcántara et al., 2009; Koudahe et al., 2022). Another consideration when planting a cover crop mixture is to carefully optimize multiple species that would enable farmers to capture a suite of benefits that wouldn’t be otherwise available if only planting one species. It is common for legumes to be mixed with grasses to reduce the C:N ratio of cover crop residues so that the decomposition process can be expedited and reduce potential nutrient immobilization (Kramer et al., 2002; Nielsen et al., 2015).

Living mulch such as cover crops, and organic mulch such as OMA, can be optimized together to maintain favorable soil temperatures and when spread on the soil surface, can reduce the germination of small seeded weed species (Iqbal et al., 2020). There is also an economic benefit to using mulches since they are often not as costly as synthetic fertilizers and allow for local materials to be delivered and recycled on site when available (Iqbal et al., 2020). One benefit from the decomposition of brassica species is that these cover crops have allelopathic effects that inhibit weed growth via glucosinolates, which aid in the suppression of weeds. Oriental mustard and hazelnut husk OMA was able to control weeds in a hazelnut orchard for 180 days after application and reduced weed dry weight by 83% at the end of the season (Mennan & Ngouajio, 2012). Other researchers found that with a CC mixture of grasses, legumes, brassicas, there was no difference in weed suppression or biomass stability compared to a single species (Florence et al., 2019). However, these researchers did find a positive correlation between biomass stability and weed suppression but believe that this is a result of their definition of diversity-productivity hypothesis in which a more diverse system should be more productive than a less diverse system due to increased resource use efficiency (Florence et al., 2019). In other cropping systems such as cereals, legume intercrops like clover have demonstrated improvements in N diversity, suppression of weeds, and increases in cereal yields in the subsequent years (Boetzl et al., 2023). The potential benefits of CC to assist in weed suppression and improve soil fertility from mixtures or single species can also be optimized when applying these crop residues in different zones of orchard systems.

1.2.3 Suitability of organic matter amendments and cover crops in orchard systems

Careful considerations for pest, fertilizer, and water management are needed when managing different zones of an orchard in two main areas, elevated berms where trees are planted and alleyways where cover crops can be planted (Figure 1). The strategy of strip management requires multiple sowing, mowing, and tilling time points for growers to accomplish multiple goals such as weed suppression and improved SOM (Bugg & Waddington, 1994). An important management practice to consider is possible competition from CC and tree crops. In an experiment studying Kura clover, it was found that this CC did not compete with pecan growth, however, the use of cool season CC like hairy vetch could suppress warm season weeds and if irrigated in the Fall, could maintain more favorable soil moisture conditions during the spring and summer (Bugg et al., 1991). Reseeding legume species and mowing strips of these CC in the same alleys annually, could build up useable N for the trees as well as help spread the seeds for germination the following year (Bugg et al., 1991). In a Gala apple orchard in Washington state, researchers also found that a legume-grass CC mixture reduced competition from water and nutrients with the main plant (Webber et al., 2022). Cover crops such as these grown in the alley can capture nitrate and release beneficial nutrients on the tree berms when using side discharge mowers (‘mow and throw’) for optimal N management.

In Midwestern USA, Kura clover was planted annually for five years in the alley of a young pecan orchard, and it was found that it acted as a sink that increased C and N inputs in the soil (Kremer & Kussman, 2011). The mineralization of N from the CC decomposition and dead microbial biomass also became available for tree uptake. In another experiment studying interactions of CC mixes of grasses, legumes, and forbs in a pecan orchard, total microbial biomass, specifically arbuscular mycorrhizal fungi (AMF), was higher in the planted CC alley compared to the tree rows (Rodriguez Ramos et al., 2022). The CC appeared to use more labile C substrates which suggested greater AMF presence that are associated with scavenging inorganic

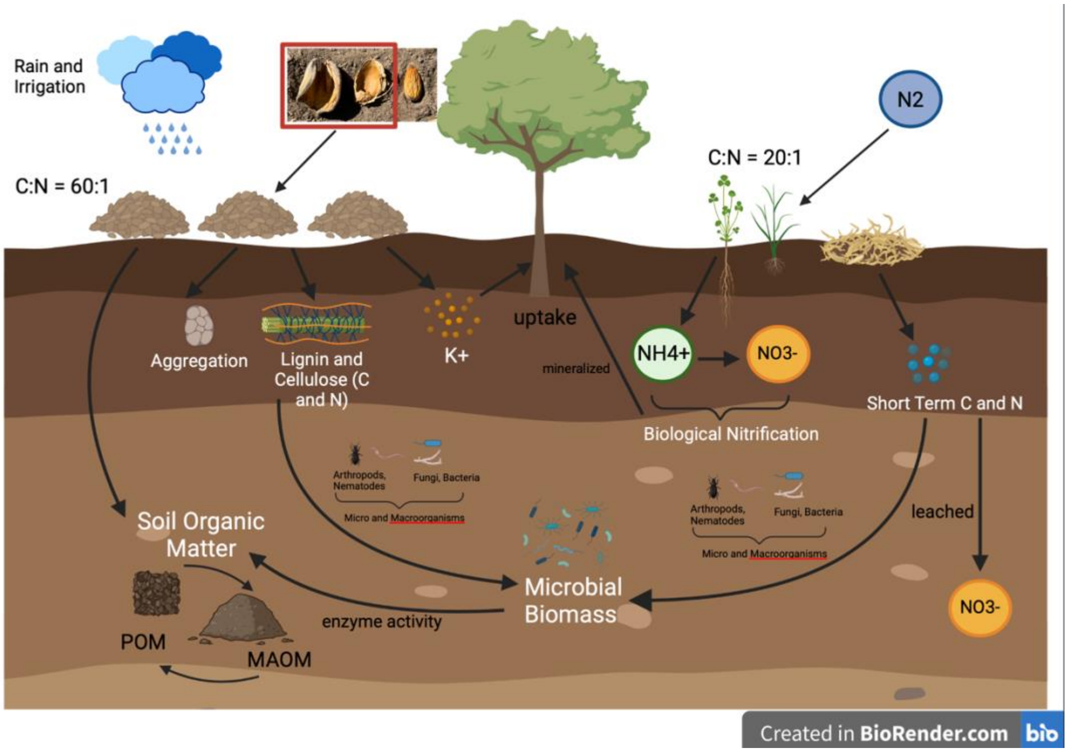


Figure 1. Potential mechanisms in the soil from surface applied organic matter amendments and grown cover crops. Almond hull and shell amendments with a high C:N ratio of ~60:1 are composed of lignin and cellulose that contribute to the formation of carbon and potassium that can be mineralized for plant uptake. The formation of soil aggregates and decomposition of these amendments also can contribute to the microbial biomass and thus the creation of soil organic matter. Seeding winter cover crops with a lower C:N ratio of ~20:1 also may contribute to the microbial biomass and can increase labile N in the soil from additional plant material, including lignin. Short-term decomposition from the cover crops may supplement mineralizable N but may also contribute to N losses through leaching.

nutrients released from SOM and litter decomposition by soil saprotrophic microbes (Chowdhury et al., 2022; Rodriguez Ramos et al., 2022).

In a Sicilian vineyard, a 10-meter wide buffer strip seeded with perennial ryegrass was monitored with a stable isotope Nitrogen-15 (15N) tracer to assess the mobility of N in the buffer and control strips. Researchers determined that the buffer strip was able to capture excess nitrate throughout the entire growing season and retain excess nitrate in rainy periods that ultimately reduced nitrate leaching (Novara et al., 2013). Cover crops act as sinks for soil N during dormant periods throughout the growing season and the removal of CC may export N away from main crops. In a maize experiment using a 15N tracer, researchers discovered that N from inorganic fertilizers was immediately available early in the growing season (Kramer et al., 2002). However, there were trends that showed the release of N from vetch residues was available 60 to 70 days after seeding, suggesting that leguminous CC can provide an incremental N supply throughout maize systems (Kramer et al., 2002). Therefore, decreasing applications of inorganic fertilizer early in the season in combination with seeding a leguminous species for sustained N release later in the season, could be sufficient to meet the N demands of a specific crop. Although using 15N to measure uptake and release of N from a specific crop does not perfectly represent a crops N demand, experiments have shown that using 15N enriched and depleted fertilizers are able to demonstrate the fate of these N sources (Chalk, 2018; Chowdhury et al., 2022; Mattos et al., 2003; Weinbaum et al., 1984). In one experiment, researchers inoculated soil cores in a greenhouse with 15N labeled substrates including plant root litter, microbial necromass, and inorganic N with plant communities that were AMF dependent compared to those without an AMF association (control) (Chowdhury et al., 2022). The researchers concluded that the AMF plant communities had lower shoot biomass than the control plants, which was to be expected since the AMF plants allocated more C to their roots. 15N uptake was greater in the shoots of AMF compared to control, most likely due to the abundance of soil microbes in these treatments. Root litter additions were the most dissimilar to the 15N additions from microbial necromass and inorganic N sources because of the higher C substrates derived from the plant roots.

Phospholipid fatty acid analyses also showed that the AMF associated plants had a high abundance of bacterial groups from Actinobacteria and Proteobacteria, suggesting shifts in microbial community composition to more bacteria dominated soils (Chowdhury et al., 2022; Wang et al., 2018). From other papers outlining N allocation and N cycling in almond trees, 15N depleted ammonium sulfate applied throughout the growing season in year 1 resulted in a two-fold higher yield the following year with increased N in the fruits as a result of 15N fertilizer applications (Weinbaum et al., 1984). In another experiment in orange trees, researchers found that 15N applied in the Spring showed 78% of all labeled 15N was allocated to the fruits across their 6-year study (Mattos et al., 2003), which supports the need for future studies to better understand the fate of N supplied by CC in conjunction with other N sources from OMA in orchard systems.

1.2.4 Soil moisture monitoring tools

To measure soil water content across time points, neutron probes, time-domain reflectometry (TDR) sensors, dielectric soil moisture sensors, and electrical resistivity tomography (ERT) are valuable tools that can be used in the field. ERT is a geophysical imaging technique that can monitor subsurface soil moisture changes over time (Kisekka et al., 2023). Researchers evaluated surface applied AHS OMA in a commercial almond orchard in Woodland, CA and their electrical resistivity data showed that the amendment improved water infiltration into the soil and improved root water uptake at depths of 0.5 and 1 meters. Other instruments to measure soil water content are by electromagnetic induction (EM) and by TDR probes. The relative low cost and noninvasive method of using EM instruments estimates volumetric water content via electrical conductivity measurements that can then be modeled over time (Huang et al., 2018). Another economically viable option is to use TDR soil moisture sensors that can measure two important characteristics, soil moisture and soil temperature (Ramos, 2017; Stevens et al., 2012). In an experiment in vineyards in Australia, amended rows with compost were measured with TDR sensors to evaluate for available soil water and total available soil water (Ramos, 2017). They concluded that an increase in available soil water at the surface and at deeper depths ranging from 20-40 cm, 40-60 cm, and 60-80 cm led to improved OM content from the compost applied.

1.2.5 Soil organic matter fractions

Soils can sequester and store large amounts of C and contain approximately two to three times the amount of C stored in vegetation and the atmosphere (Gross & Harrison, 2019). Therefore, reducing C loss amounts of soil organic carbon (SOC) can help suppress greenhouse gas emissions. SOC is commonly used to measure soil health and has been defined by two pools of C, particulate organic matter (POM) and mineral associated organic matter (MAOM) (Angst et al., 2023; Liptzin et al., 2022). To predict soil C in these pools, research has focused on C mineralization in POM and C storage in MAOM. However, increased soil microbial responses in soil C cycling also have important implications for C storage resulting in more mineralized C and therefore increased amounts of SOC. Deciding which soil health indicators to measure C dynamics can be challenging, but the broad area of focus will be on C cycling in POM and MAOM. Two indicators to measure these general C pools are permanganate oxidizable C (POX-C) and water extractable organic C (WEOC) and are easily catabolized by microbes. Other common indicators used to measure soil health are ß-Glucosidase enzyme activity, which can represent C cycling dynamics, potential C mineralization for 24 or 96 hours that provides an assay in the laboratory for C compounds metabolized by microbes, and total and individual microbial biomass (Liptzin et al., 2022; Rodriguez Ramos et al., 2022). Variability when measuring soil samples for C depends on spatial, temporal, and analytical conditions such as seasonal changes that may affect the presence or absence of microbes when taking samples for PLFA microbial biomass analyses (Liptzin et al., 2022). To reduce this variability, researchers suggested sampling at recurring time points and taking adequate samples to capture potential variability in the field. SOC storage is also greater in deeper soil layers and sampling at lower depths may also explain variability compared to the surface (Currie et al., 1996; Liptzin et al., 2022; Núñez et al., 2022; Rovira & Vallejo, 2002).

MAOM research has focused on its ability to store C from the formation, accumulation, and chemical composition of this C pool. One thought is that MAOM formation can be manipulated by increasing microbial biomass and necromass using plants with high litter quality such as legumes (Angst et al., 2023). Other studies have argued that the formation from MAOM is separate from POM, but that dissolved compounds from leaf litter can be directly metabolized by the microbial community (Angst et al., 2023; Xu et al., 2022). Although MAOM and POM are two distinct pools of C, their roles are linked in addressing soil health benefits. POM is mostly derived from partly decomposed plant material and has a short residence time which allows for quicker decomposition depending on management and environmental factors. MAOM can persist for thousands of years and is occluded within small microaggregates, which has a potential for C sequestration (Angst et al., 2023). However, there is also a potential for POM to contribute to C sequestration depending on soil conditions in grasslands or in semiarid environments that have a limited ability to protect against mineralization (Angst et al., 2023; Bullard & Smither-Kopperl, 2020; Fenster et al., 2023). In an incubation experiment comparing differences from semiarid dryland and irrigated maize, researchers studied the effects of irrigation on POM and MAOM formation from maize litter in this no tilled system (Núñez et al., 2022). Researchers from Colorado State University discovered that most of the C formed from the maize litter OM was found in the MAOM pool which was to be expected. They also discovered that irrigation did not have an effect on the amount of C formed in the macroaggregates. This was thought to be a result of a higher proportion of C in the macroaggregates compared to a lower C proportion as free MAOM from the maize litter (Núñez et al., 2022). Although C inputs were almost five times greater in the irrigated than in the dryland treatments, higher litter decomposition does not always equate to greater SOM formation. Consequently, there may be C losses through microbial respiration from large amounts of litter that have formed from new SOM sources (Liptzin et al., 2022; Núñez et al., 2022) that have not been well studied as a soil health indicator.

1.2.6 Soil microbial community functions

Fungi and bacteria are the primary organisms that build SOM with fungi dominated soils accumulating more C than bacteria dominated soils because fungi produce a larger fraction of recalcitrant C for long-term storage (Jackson et al., 2017). Fungi decomposers can sequester C and form soil macroaggregates, but their role in determining the net effects of C sequestration is not well understood. However, other researchers suggest that a soil continuum model that focuses on the ability of organisms to access SOM via a range from intact plant material to highly oxidized carbon, would better explain the functions of decomposers across multiple time points (Gross & Harrison, 2019; Lehmann & Kleber, 2015). An important component of SOM is the soil microbial biomass, which is the labile portion of the soil organic fraction that constitutes 1-3% of the total soil C and 5% of the total soil N (Horwath & Paul, 1994). Soil microbial biomass is one indicator that can assess soil fertility in different management practices such as tillage, crop rotations, and nutrient activity. OMA from plant litter is a natural process in forest systems and in orchard crops that contribute to the formation of SOM. The decomposition of aboveground plant litter has two mechanisms that form SOM. First, non-structural compounds such as lignin and cellulose are quickly transferred from the soil as dissolved organic matter (DOM), which adsorb to minerals or is used by microbes resulting in the formation of mineral associated organic matter (MAOM) (Marschner & Kalbitz, 2003; Núñez et al., 2022; Xu et al., 2022). The other mechanism is by a physical transfer of particulate organic matter (POM) as structural compounds that are transferred into the soil when plant residues are physically disturbed by environmental conditions (Núñez et al., 2022). Although maintaining ground cover is an important component to improving soil health, high litter decomposition may not lead to proportional increases in SOM formation because of unforeseen anaerobic conditions that reduce microbial C use efficiency (Jackson et al., 2017; Núñez et al., 2022). OMA with a high carbon to nitrogen (C:N) ratio may also have adverse effects in soils highly saturated with C. The increased C input may accumulate the formation of POM and reduce the formation of MAOM, which is typically associated with C storage (Angst et al., 2023).

1.3 Harvesting methods to build the soil organic layer

Improving air quality during almond harvest is one of the Almond Board of California’s goals to achieve by the year 2025. The conventional almond harvest timeline begins with shaking the tree, on-ground drying, sweeping, picking up the dried fruits and then placing them in neat windrows in the alleyways (Micke, 1996). The current on-ground harvest method creates large amounts of dust during the sweeping and picking up phase which contributes to pollution and dust inhalation from airborne particles. In the San Joaquin Valley, visible dust opacity ranged from 23.3% to 29% with conventional on-ground harvesters, compared to low dust harvesters with an average dust opacity of 13.94% (Arzadon et al., 2023). Another limitation is that on-ground harvest relies on keeping the shaken nuts on a dry orchard floor for multiple days, which can serve as a breeding ground for pest infestation (Chen et al., 2021). However, off-ground harvest has the potential to reduce dust and pest damage, along with implementing alternative approaches such as early harvest (Smith et al., 2022) and maintaining ground cover in the alleyways. Off-ground harvest equipment utilizes a catch frame to collect the shaken nuts so that they can be dried in offsite commercial dryers, stockpiles, or mechanically dried with hot air (Chen et al., 2021). In an experiment studying early almond harvest, in two sites in the Central Valley, researchers discovered that there was a 76% reduction in navel orangeworm (NOW) infestations when nuts were harvested three to four weeks earlier than standard harvest (Smith et al., 2022). The combined efforts of using off-ground harvesters and early timing approaches will not only reduce typical harvest steps but will contribute to sustainable pest management practices.

Off-ground harvesting eliminates both the sweeping and blowing steps that traditional on-ground harvesters use to place shaken nuts into windrows. It also gives growers more flexibility in how they manage their orchard floor and when they plan to harvest. Studies evaluating OMA in almond orchards have shown that building SOM over time through yearly applied amendments, increased net N mineralization compared to soils with no previous OMA history (Andrews et al., 2021; Hartman et al., 2024). Building OMA and crop residues over time with the use of off-ground harvesters would encourage diverse management strategies that include multiple CC mixes, integrated crop livestock, and specific irrigation scheduling.

1.4 Current knowledge of organic matter amendments and cover crop use in almond systems

Almonds produced in California have approximately a 2:1 ratio of hulls to kernels and a 0.7:1 ratio of shells to kernels. In 2017, nearly 1.5 million metric tons of almond hulls and 0.6 million metric tons of shells were produced based on dry weight calculations. Most huller and sheller processors separate soft shell variety hulls (Nonpareil and other pollinizers) from the semi-hard/hard shell varieties (Butte, Fritz) because these prime hulls provide sources of carbohydrates for dairy cows (Huang & Lapsley, 2019; Swanson et al., 2021). The almond hulls and shells (AHS) produced in the Northern Central Valley, present an opportunity for local California almond growers to utilize these byproducts as OMA since the additional transportation costs of AHS as cattle feed and bedding to the Southern Central Valley are not economically viable (Sumner et al., 2014). In a grower analysis paper, survey responses indicated that 51% of OMA users and 48% of OMA nonusers deemed soil biology as the primary OMA benefit. Water holding capacity (WHC) was surveyed as the least important OMA benefit (Khalsa & Brown, 2017). Researchers proposed that more growers were interested in improving their soil microbial diversity and were less convinced that OMA has the potential to improve WHC.

In a 40-year-old avocado orchard in Spain, almond shells were applied to trees and were compared to unamended trees to assess for microbial diversity and *R. necatrix*, a soil borne phytopathogen in avocado that causes white root rot (Vida et al., 2016). Researchers concluded that almond shell amended trees had increases in the abundance of *Ascomycota*, the largest group of fungi, which suggests that these fungi have a high capacity for C degradation based on these microbial shifts in the treated soil. *R. necatrix* was also significantly lowered in the amended trees which supported their hypothesis that almond shell OMA served as a pathogen suppressant. In a sweet pepper greenhouse experiment studying microbial diversity comparing almond shells and almond hulls as OMA, researchers found that water soluble organic C was greater in hulls than in the shells (Valverde et al., 2013). This result led them to believe that the almond hull OMA increased microbial biomass due to higher bulk density values during the decomposition process.

The almond hull treated sweet pepper plants also had approximately 10% greater yields compared to the almond shell treated plants.

In a 16-year almond orchard study in California, regenerative and conventional management practices were compared to evaluate for overall soil health, biodiversity, yield, and profit (Fenster et al., 2021). Regenerative was defined by the use of OMA, no-till, prescribed grazing, maintaining ground cover using cover crops or resident vegetation and planting hedgerows. OMA in regenerative orchards with no-till, had 31% more SOC and 16% more total soil nitrogen than conventional orchards in the top 20 cm of soil (Fenster et al., 2021) which aligns with other studies (Núñez et al., 2022; Ramos, 2017). Most soil indices used that included soil carbon, micronutrient levels, water infiltration rates, and soil health were all improved in regenerative orchards. Using cover crops and OMA as regenerative practices have been well studied singularly, but combining these practices together could have profound benefits for soil biodiversity. Managing orchards with multiple ecological goals including cover crops and OMA in California are not the primary goals of growers, but there is a growing interest to implement some of these regenerative practices (Khalsa & Brown, 2017). In a review on the current knowledge and barriers to entry for cover crop use in California almond orchards, the focus on cover crop roots, aboveground vegetative biomass, and flowers are addressed (Wauters et al., 2023). Seeding cover crops in almond orchard alleys can improve water infiltration and can be irrigated by rainfall during the winter season in Mediterranean climates. Another benefit from CC can reduce navel orangeworm (NOW) disease if “mummy” (remnant) nuts are shaken and more readily decomposed in CC areas. Most California almond growers rely on honeybees to pollinate the almond flowers during late winter. Increasing CC diversity and timing may incorporate wild bee populations and other pollinators during flowering periods when the CC is in bloom, thus reducing the need to rely on pollination services. The wide variety of CC and OMA available offer promising solutions to maintain ground cover, improve soil management, and diversify ecological landscapes in orchard systems (Alcántara et al., 2009; Khalsa et al., 2022; Khalsa & Brown, 2017; Lepsch et al., 2019; Repullo-Ruibérriz de Torres et al., 2021).

1.5 Conclusion

Much progress has been made in understanding current research questions about soil functionality in relation to organic matter amendments and cover crops. Improving soil health in orchards, studying nitrogen and carbon dynamics, practical timing of OMA and CC applications, and strategizing farming goals will help to address knowledge gaps for future research and contribute to more sustainable farming practices in almond. Decision making when choosing types of cover crop species to achieve dense aboveground biomass, weed suppression, or nutrient supplementation needs to be carefully considered especially when combined with surface mulch, compost, or nutshell amendments. Altogether, continuing to better understand the functionality of soil microbes in specific agricultural environments across growing seasons can provide growers with useful management tools when considering implementing regenerative practices.

Chapter 2: Soil health outcomes of surface applied almond hull and shell amendments and cover crops

2.1 Introduction

Ensuring global food security is threatened by global warming, soil degradation, and intensive management practices (Ory et al., 2022; Prăvălie et al., 2021). Global soil erosion has increased by 2.5% between 2001 and 2012 which highlights the need to implement soil management strategies such as utilizing soil amendments to support healthy living soils and their nutrient cycling, and water retention functions (Broschat, 2007; Ory et al., 2022; Prăvălie et al., 2021; Vida et al., 2016). Mulches and recycling crop residues such as the outer shells and husks from nuts, have been shown to reduce soil compaction, improve SOM, and support profitable crop production in perennial agroecosystems (Chalker-Scott, 2007; Fenster et al., 2021). Thus, implementing these practices and emphasizing their effectiveness in almond orchards in particular will help to close the production-waste loop.

Previous research studying the effects of almond hulls and shells (AHS) as organic matter amendments (OMA) in almond orchards from 2019-2022 aimed to utilize these byproducts that consisted of 1.6 billion pounds of hulls and 1.8 billion pounds of shells from the crop year in 2022 (Almond Board of California, 2022). Researchers reported that 68 kg of nitrogen (N) and 75 kg of potassium (K), the two macronutrients required for photosynthesis and plant growth, were removed in hulls and shells with harvest based on 1000 kg of kernel almond yield and are the (Muhammad et al., 2018). Therefore, returning nutrients back to the orchard with AHS can supply as much as 90 kg of K to offset or supplement fertilizer applications (Andrews & Kassama, 2022). In addition to K rich amendments, cover crops (CC) can supply N back to trees with an assortment of species mixes including warm and cool season CC or self-reseeding species with N rich benefits (Bugg et al., 1991; Devi, 2021; Novara et al., 2013).

Additional regenerative methods during the almond harvest season are being tested with the use of off-ground, or synonymously referred to as catch-frame harvesters. Typical almond orchards leave the soil bare which can degrade the soil, decrease soil moisture, and reduce microbial diversity. However, catch-frame harvesters would allow for surface applied OMA and CC to be planted without disturbing the topsoil and provide an alternative management strategy to enhance orchard biodiversity. Dust reduction and pest disease are also added benefits of off ground harvest that can support sustainable practices in California almond orchards. Maintaining crop residues and OMA yearly will also help to build soil organic matter (SOM) over time and can have compounding benefits if these amendment layers remain undisturbed with catch-frame harvest equipment.

To address the impacts of bare soil cover on soil health, we tested two regenerative practices in an almond field trial that included broadcasting AHS and planting a CC mix in combination with off-ground harvesting. The objective of this experiment was to evaluate soil health by measuring soil physical, chemical, and biological properties under AHS OMA and assess the short-term nutrient status in the soil with the introduction of a CC mix. To quantify the carbon and nitrogen content in the CC, aboveground biomass samples collected will provide a baseline for AHS OMA and CC interactions in this study. Research questions to be answered are as follows: (1) How do soil physical properties and microbial community composition under AHS OMA shift with on and off-ground harvest in the tree row after two years of treatment application? (2) How do yearly fall applied AHS OMA impact CC biomass, nutrient status, and short-term decomposition of AHS in the following Spring? We hypothesized that the previous two-year surface applied AHS OMA in the tree rows will increase soil mean weight diameter of stable aggregates and C pools in particulate organic matter (POM) due to the immediate mulch layer maintained in the off-ground treatment. Mineral associated organic matter (MAOM) is mostly formed by microbial decomposition and soil PLFA analyses from previous years indicated that both fungi and bacteria biomass have increased, which could drive the formation of MAOM and therefore building SOM over time. Additional benefits from AHS OMA include improving soil pH, CEC, and increasing K plant status although yield benefits are not guaranteed at the current year three of this trial. We also hypothesized that half of the AHS OMA will decompose approximately five months from the initial application date, releasing most of its K in the first six and a half weeks (Andrews & Kassama, 2022). CC biomass will decompose faster than AHS OMA once terminated, due to its lower C:N ratio and will release N more quickly in the alleyways but will have reduced aboveground growth due to previous AHS OMA applications that may suppress the full growth potential of the CC. AHS OMA and CC biomass will both be maximized with the use of off-ground harvest to maintain the organic layer when compared to on-ground harvest that sweeps away this beneficial soil building block.

2.2 Materials and Methods

2.2.1 Site Description

The field trial, Westwind Farms, located in Woodland, California in the Sacramento Valley is a 62 hectare (152 acre) mature commercially operated almond orchard in its 10th year. Our trial is in the southwest corner of the orchard totaling 10 hectares (25 acres). Every other tree row is a Nonpareil variety on Bright Hybrid 5 rootstocks with alternating pollinizer tree rows of Monterey, Carmel, and Butte varieties. Trees are spaced 4.6 x 6.7 meters (15 x 22 ft) and are irrigated with micro-sprinklers. The soil type in our trial is a San Ysidro loam (USDA NRCS, 2019). Prior to treatment applications in 2020, the average soil pH was 7.4, average SOM was 2.3%, and average CEC was 20 meq 100 g^{-1} in the top 0-10 cm soil. Each Fall, the grower applied 2.2 metric tons ha^{-}

^{1} (1 US ton ac^{-1}) of compost throughout the entire orchard as a best management practice, prior to AHS amendment application. This field experiment focused on studying historical effects of AHS OMA and short-term interactive effects of planted CC in alleyways on decomposition, nutrient cycling, and N and C release. AHS amendments were applied on 10/7/2020, 10/4/2021, and 10/7/2022. October 2020 and 2021 application was applied by a previous PhD student, Ellie Andrews, and is mentioned here to show historical AHS applications for this site as recommended rates for the most recent Fall 2022 application. In Fall 2020, AHS (32% hulls and 68% shells) were broadcast applied in the tree rows and in the alleyways at a rate of approximately 18 tons ha^{-1} (8 fresh US tons ac^{-1}) with 6.6% moisture in the mix resulting in 16.8 dry tons ha^{-1} (7.5 dry US tons ac^{-1}). In Fall 2021, AHS (53% hulls and 47% shells) were applied with a side spreader to tree rows only at a rate of approximately 18 tons ha^{-1} (8 fresh US tons ac^{-1}) with 2.1% moisture in the mix resulting in 17.6 dry tons ha^{-1} (7.8 dry US tons ac^{-1}). In Fall 2022 (25% hulls and 75% shells), AHS were broadcast applied in the tree rows and in the alleyways at a rate of approximately 18 tons ha^{-1} (8 fresh US tons ac^{-1}) with 5.8% moisture in the mix resulting in 16.9 dry tons ha^{-1} (7.5 dry US tons ac^{-1}). Resident vegetation was allowed to grow in the alleyways (2020-2021) and the first year seeding a CC mix occurred in Fall 2022 to analyze potential interactions between AHS OMA and CC.

2.2.2 Experimental Design and Conditions

This trial was implemented as a randomized complete block design (RCBD) with four treatments replicated over four blocks. The four treatments studied were a control treatment without cover crop and without AHS with on-ground harvest, cover crop treatment with off-ground harvest, AHS amendment treatment with on-ground harvest, and AHS amendment treatment with off-ground harvest. Each treatment was applied to entire rows from tree 1 to tree 40 with a total of

30 tree rows in the RCBD. AHS OMA were broadcasted to both sides of the tree row in the berms (over the tree roots) and in the alleyways with a compost spreader. On 10/07/2022 the fresh AHS mix had an average moisture of 5.8% and was applied approximately at a rate of 18 tons ha^{-1} (8 fresh US tons ac^{-1}). The AHS mix consisted of approximately 25% hulls and 75% shells. The berm area was approximately 36% of the total ground area in the orchard and the alleyway was approximately 64%. On 11/28/22 a CC mix of 60% peas (spring forage), 35% oats, and 5% yellow mustard (*brassica juncea*) was seeded in a 3-meter (10-ft) strip in the center alleyway at the recommended rate of 112 kg/ha (100 lb/ac). CC were seeded on both east and west alleyways totaling 8 alleyways and CC were terminated by flail mower on 4/26/23.

Treatment Abbreviations

Treatment name and abbreviation.

Treatment Number	Treatment Name
T1	Control On-Ground Harvest (Unamended)
T2	Off-Ground Harvest (Unamended)
T3	Amended On-Ground Harvest
T4	Amended Off-Ground Harvest

Soil treatment name and abbreviation.

Treatment Number	Treatment Name
T1	Control Soil (On-Ground Harvest)
T2	Cover Crop Catch Frame Soil
T3	Amended Soil (On-Ground Harvest)
T4	Amended Catch Frame Soil

AHS nutrients

Samples from the initial AHS amendment application in Fall 2022 were oven dried at 60 ℃, ground, and submitted to the UC Davis Analytical Laboratory for nutrient concentrations via nitric acid digestion that included nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, zinc, manganese, iron, and copper by inductively coupled plasma atomic emission spectrometry (ICP-AES) (UC Davis Analytical Lab, 2024). Litter bags are typically used in agroforestry studies to measure short to long term decomposition and have been utilized to assess for residue retention from surface applied amendments in agricultural experiments (Leal et al., 2023; Sari et al., 2022). Immediately after AHS application on 10/17/22, 32 litter bags (20x20cm) were filled with 95 grams of oven dried AHS and installed in the center of alleyways to measure decomposition trends over time. Square litter bags were made of 1/32-inch nylon mesh (Memphis Net and Twine Company) and were filled with AHS based on calculations of alleyway area, application rates, and percent moisture of fresh applied AHS. AHS litter bags were collected before CC termination on 4/25/23 (192 days after AHS application), oven dried at 60 ℃, and dry mass was recorded. To simulate effects of CC decomposition in the AHS off-ground treatment, 32, 20 cm by 20 cm square litter bags (Florence et al., 2019) filled with approximately 70 grams of oven dried CC biomass were installed in the center alleyways of the CC treatment and AHS off-ground treatment rows on 4/28/23. On 7/26/23 (90 days after CC termination), CC litter bags were removed, oven dried at 60 ℃, and dry mass was recorded.

Soil health indicators

Prior to Fall amendment application, baseline soil samples were collected on 9/13/22 from all treatments in all four blocks at depths of 0-10 cm, 10-20 cm, and 20-30 cm within a 24-inch circumference of the tree base (approximately halfway between the micro-sprinkler and the base of the tree) in the tree rows (Figure 2.1). Soil samples were measured for baseline soil fertility status by inductively coupled plasma atomic emission spectrometry (ICP-AES) that included: nitrate-nitrogen (nitrate-N), Olsen phosphorus (Olsen-P), exchangeable potassium (XK), exchangeable sodium (XNa), exchangeable calcium (XCa), exchangeable magnesium (XMg), cation exchange capacity (CEC), SOM percent by the Walkley-Black Method, and pH by saturated paste extract (UC Davis Analytical Lab, 2024). Three subsamples were taken from each treatment row for each depth, air-dried, and aggregated for each experimental unit prior to submitting for analysis at the University of California, Davis Analytical Lab.

Soil samples for bulk density, aggregate stability, particulate organic matter (POM), and mineral associated organic matter (MAOM) measurements were collected on 9/13/22 and sampled in the control, AHS on-ground, and AHS off-ground treatments. All soil samples were collected using an 8.3 cm diameter by 6 cm tall metal ring and mallet and soil were air dried. Soil wet aggregate stability was measured with an automatic soil sieve with an overhead rainfall simulator (Fritsch Analysette 3 Vibratory Sieve Shaker) (Kemper, 1965). All aggregate stability samples were prepared by passing through an 8 mm mesh sieve and then prepared further by separating into 30-gram subsamples. Each subsample was evenly spread across a stack of three mesh sieves beginning with the largest to the smallest sieve size 1) >2 mm (large macroaggregates) 2) 250 µm (small macroaggregates) 3) >53 µm (microaggregates) 4) <53 µm (small microaggregates) (Six et al., 2000). Each sieve was removed after the water ran clear (~15-30 seconds) and the remaining soil aggregates on the sieve were gently sprayed with deionized water from a wash bottle to capture all small aggregates into labeled aluminum disposable loaf pans of size 8.5 by 4.5 inches. All samples in pans were placed into a 60 ℃ oven for one to two weeks or until weights stabilized.

Soil organic matter fractions (POM and MAOM) were weighed to 10 grams. Each 10-gram subsample was put into falcon tubes with 30 ml of 5% sodium hexametaphosphate and five, 4 mm glass beads, and put on an oscillating shaker for 18 hours. After shaking, the soil slurry samples were placed on a stack of mesh sieves beginning with the larger size >53 µm (POM) and then following the smaller size <53 µm (MAOM) with an overhead rainfall simulator (Fritsch Analysette 3 Vibratory Sieve Shaker) and shaken until the water ran clear (~ 30 seconds). All samples were placed into a 60 ℃ oven for one to two weeks or until weights stabilized (Jilling et al., 2020; Midwood et al., 2021). Floating debris and AHS pieces were removed before placing the samples into the oven. Aggregate stability, POM, and MAOM samples were transferred from loaf pans and encapsulated into tin capsules for total C and N analysis via combustion method at the University of California, Davis Stable Isotope Laboratory. Mean weight diameter (MWD) was calculated by averaging the sum of the products of aggregate fractions and the mean diameter of each aggregate sieve, excluding floating material, to determine the stability of soil aggregates in the three treatments sampled.

The aggregates remaining on each sieve were dried and weighed using the following equation:

$$MWD = \sum_{i=1}^{n} \overrightarrow{x_i} w_i$$

Where $x_i^{\rightharpoonup}$ is the mean diameter of the sieve size the wet stable aggregates did not pass through and w_i is the ratio of stable aggregate weight to the total weight for each sieve fraction measured (Rieke et al., 2022).

Soil microbial analysis for coarse functional group community composition analyzed via phospholipid fatty acid (PLFA) were collected on 5/15/23 in the control and the AHS off-ground harvest treatments in the tree rows sprinkler zone. Three subsamples were taken from the control and the amended off-ground treatments in all four blocks totaling 24 soil samples. The AHS amended samples were scraped back before sampling so only soil was collected. Samples were placed into Ziplock bags, packaged on ice in a styrofoam cooler, and immediately shipped overnight to Ward Laboratories (Kearney, NE) for analysis.

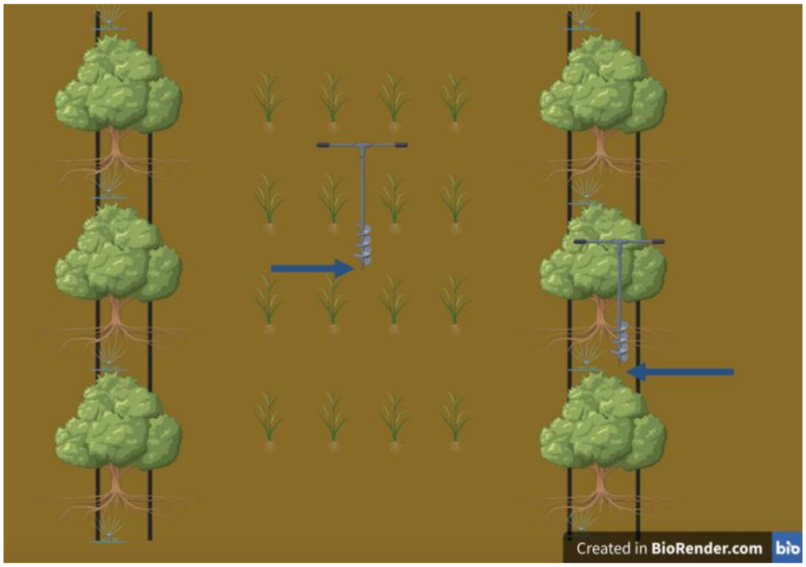


Figure 2.1. Sampling locations in the tree berms and alleyways for soil, Westwind site.

Soil moisture monitoring

Acclima Time Domain Reflectometry (TDR-315 N) soil moisture sensors were installed in the control and amended off-ground treatments in tree rows with the Acclima Solar DataSnap SDI-12 Data Logger. Installation occurred on 2/25/23 with three soil moisture sensors and one data logger per treatment and block, totaling 24 TDR probes and 8 dataloggers measured monthly from March through August 2024. The TDR sensors measured volumetric moisture (percent), temperature (°C), permittivity (ε, the ability to hold an electric charge), conductivity (µS cm^{-1}), and pore water electrical conductivity (PWEC, µS cm^{-1}). All Acclima TDR sensors were installed approximately 0.91 m (3 ft) from each micro-sprinkler, halfway between the micro-sprinkler and the edge of the irrigation zone at three locations: in the center of the row between the sprinkler and the north tree, toward the alley directly east of the sprinkler, and on the northeast diagonal between the other two sensors (Figure 2.2). Soil moisture data were downloaded on 5/3/23, 7/18/23, and 8/8/23 and all sensors were removed on 8/8/23 before harvest to prevent equipment damage.

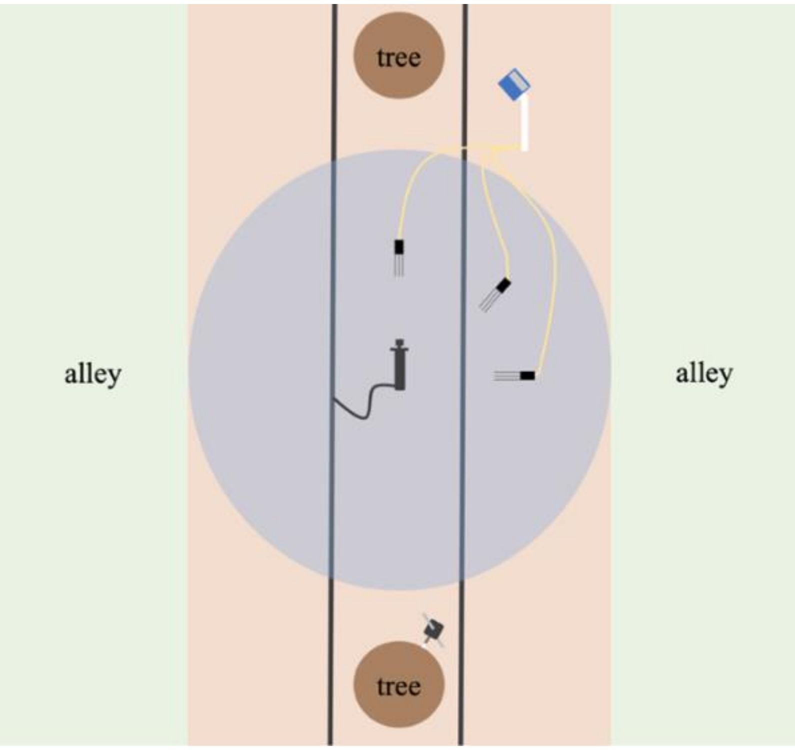


Figure 2.2. Acclima TDR soil moisture sensor locations in north, northeast, and east directions of the sprinkler head, Westwind 2023 (Andrews, 2022).

Cover Crop biomass and nutrient uptake and release

Prior to mowed CC termination on 4/26/23, 0.5 m^2 quadrats were created to collect CC biomass samples and were randomly placed in the center alleyway approximately halfway between each tree row. 4 aboveground CC biomass samples were collected and separated by each species within 2 subplots for each of the 4 blocks. 2 aboveground resident vegetation (weeds) samples were collected within 2 subplots for each of the 4 blocks. Biomass samples were air dried at 60 ℃ for two to three days, and their dry mass was recorded. Plant samples were ground to pass through a 1 mm sieve and submitted to the UC Davis Analytical Laboratory for nutrient analysis via nitric acid digestion that included N, P, K, S, B, Ca, Mg, Zn, Mn, Fe, Cu, and C. Average height in cm were recorded in each quadrat sample and three subsample soil cores at 0-10 cm depths were aggregated and a final 8 soil samples were submitted to the UC Davis Analytical Laboratory after aboveground biomass had been removed. CC treated soil samples were collected in the alleyway at 0-10 cm depths, (Figure 2.2) for a soil fertility analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES) that included nitrate-nitrogen (nitrate-N), Olsen phosphorus (Olsen-P), exchangeable potassium (XK) via ammonium acetate extraction, exchangeable sodium (XNa), exchangeable calcium (XCa), exchangeable magnesium (XMg), cation exchange capacity (CEC), SOM percent by the Walkley-Black Method, and pH by saturated paste extract (UC Davis Analytical Lab, 2024).

Almond yield

Yield data for each tree row was collected by harvest machinery in collaboration with on-ground and off-ground equipment operators. A traditional shake and catch harvester was used in the on-ground treatment rows. A catch-frame harvester was used in the off-ground treatment rows which placed the shaken nuts into the center alleyway while eliminating the sweeping step in the tree rows. All yield data for individual tree rows were collected by mechanically sweeping, picking up, and placing the nuts into a Thomas nut weigh cart. Fresh weight yield subsamples of at least 2 kg were collected from each plot, weighed fresh, oven-dried, and weighed at dry weight to calculate for percent moisture. Dry whole fruits were then separated from debris (sticks, leaves, remaining hull/shell amendments, etc.), and subsamples were weighed. The hull and shell trash were weighed separately from the remaining trash. 50 dry whole fruits were separated into kernels, hull, and shells and weighed. Crack out percentage was calculated as follows:

$$Crack\ out\ \% = \frac{dry\ mass\ of\ 50\ kernels}{dry\ mass\ of\ 50\ whole\ fruits} * 100$$

For 40 trees within each plot across the field trial, kernel yield was calculated using dry kernel weight for the number of trees divided by the area covered:

$$Kernel\ yield = (dry\ mass\ kernels * 40\ trees * 16\ rows)/(area\ coverage)$$

Statistical Analysis

Data was analyzed in R (R version 2023.06.1+524 (2023.06.1+524)). Data visualization was performed using the package ggplot2. Soil data was performed using analysis of variance (ANOVA) and means were compared using Tukey multiple comparisons of means in R. Linear models were used with treatment as the fixed variable and block as the random variable when compared at a specific time point. When compared across multiple time points, the interaction of treatment*length of time was included, and plot as the random variable to account for repeated sampling from the same plots over different periods of time. Model assumptions of normality and similar variances were tested using the Normality and Quantile-Quantile plots from the performance package in R. Combined soil data and evaluation of directional shifts of microbial community composition as environmentally constrained by fertility and physical variables was analyzed using Canonical Correspondence Analysis (CCA). For response variables where subsamples were not aggregated prior to analysis (e.g. cover crop biomass, PLFA), plot was included as a random effect nested within block. Alpha values were all set to 0.05. Compact Letter Display (CLD) groupings were performed using the estimated marginal means (multcomp package) for multiple pairwise comparison (Tukey method) after ANOVA analyses were performed. Linear regressions were performed using ggplot2 for decomposition over time.

2.3 Results

2.3.1 Baseline soil fertility

Prior to treatment establishment in Fall 2022 and after 2 years of applied AHS OMA, XK was significantly higher in the amended treatments than the two unamended treatments (Table 2.1). The pH values were significantly higher in the unamended off-ground treatment compared to the amended on-ground treatment. Organic matter trended higher in the amended treatments compared to the unamended treatments, although not significant. Average Olsen-P was slightly lower in amended treatments, although not significant. Soil samples indicated that the largest significant soil XK increased in the amended treatments compared to unamended treatments (Table 2.1). Cation Exchange Capacity (CEC) was slightly higher in the amended off-ground treatment with no significant differences between the other three treatments. Average pH was significantly higher in the unamended off-ground treatment compared to the amended on-ground treatment.

Table 2.1. Fall baseline soil samples at a depth of 0-10 cm were collected on 9/13/22 at Westwind in the control on-ground soil (T1), unamended catch frame soil (T2), amended on-ground soil (T3), and amended catch frame soil (T4). Letters indicate significant differences between treatments using a threshold of p=0.05.

Treatment	NO3-N (ppm)	Olsen-P (ppm)	XK (ppm)	XNa (ppm)	XCa (meq 100g-1)	XMg (meq 100g-1)	CEC (meq 100g-1)	% OM	pH
T1: Control On-Ground Soil	2.25	3.23	125.17 a	1355.75	11.25	4.11	21.58	1.78	7.83 ab
T2: Unamended Catch Frame Soil	1.57	3.45	116.17 a	1334.92	12.58	4.50	23.18	1.87	7.94 b
T3: Amended On-Ground Soil	1.89	2.55	200.67 b	1365.17	11.23	4.23	21.91	2.01	7.62 a
T4: Amended Catch Frame Soil	1.74	2.35	182.58 b	1254.58	12.53	4.54	23.01	1.97	7.85 ab

2.3.2 Initial nutrients (hull/shell and soil) before AHS application

Initial average AHS amendment nutrient concentrations are provided in Table 2.2 and 2.3. K concentration of AHS was approximately 1.89% with an average C:N ratio of 64:1. At the given rate, AHS application supplied more macronutrients than micronutrients.

Table 2.2. Average applied nutrient concentrations in hull/shell mix and rates at Westwind, 10/7/22. The hull/shell mix was 25% hulls and 75% shells.

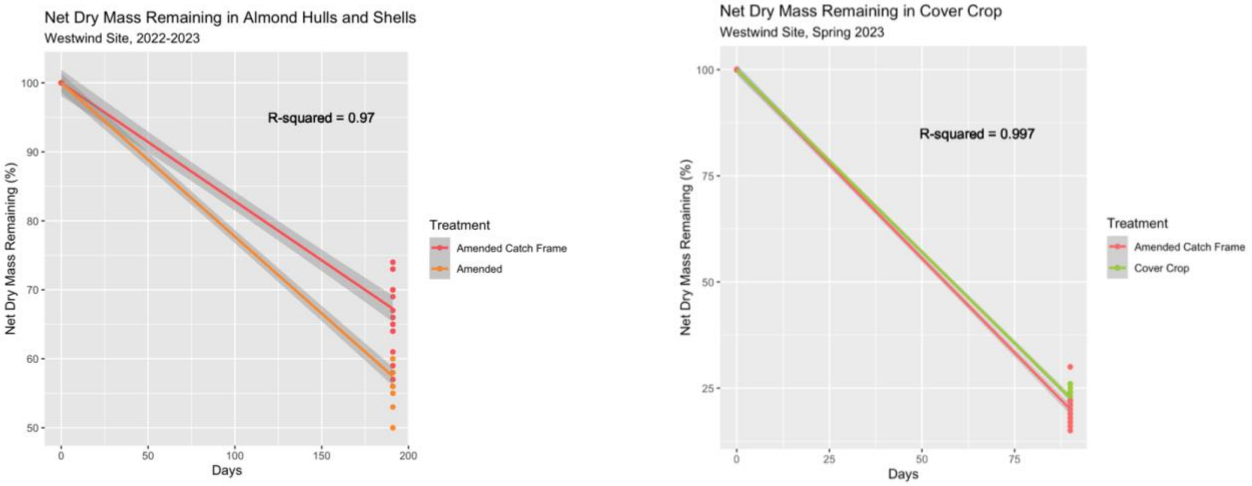
Application Date: 10/7/22												
	(%)						(ppm)					
C:N	C	N	P	K	Ca	Mg	S	B	Zn	Mn	Fe	Cu
64:1	47.1	0.745	0.07	1.89	0.253	0.081	401.3	73.1	7.9	16.4	401.6	3.95
Nutrient rate applied 16,812 dry kg/ha												
--	11130	176	16	447	59	19	94	17	1.86	3	94	0.9

Table 2.3. Average hull/shell amendment nutrient concentrations sampled 201 days after application. Letters indicate significant differences between sampling time points, using a threshold of p=0.05.

Nutrient	Initial Time: 10/7/22	Final Time: 4/25/23
N (%)	0.745 a	0.927 b
P (%)	0.0700 b	0.0495 a
K (%)	1.894 b	0.107 a
S (ppm)	401 a	982 b
B (ppm)	73.1 b	20.8 a
Ca (%)	0.253 a	0.577 b
Mg (%)	0.0810	0.0922
Zn (ppm)	7.89 a	62.60 b
Mn (ppm)	16.4 a	55.4 b
Fe (ppm)	402 a	2218 b
Cu (ppm)	3.95 a	6.87 b
C (%)	47.1 b	45.4 a
C:N Ratio	64.5 b	49.4 a

2.3.3 Amendment and cover crop decomposition

192 days after litter bag application, 62% of the average AHS dry mass remained and 38% of the material decomposed (Table 2.4) (Figure 2.3a).



(a) (b)

Figure 2.3a and b. Decomposition of almond hull and shell amendment and decomposition of cover crop mix expressed as percent net dry mass remaining in linear regression, Westwind 2022-2023.

The dry mass remaining in the amended catch frame treatment was slightly higher compared to the amended on-ground harvest treatment, although not significant. Ninety days after CC termination and installation of the CC litter bags, 31% of the average CC dry mass remained and 69% of the material decomposed (Table 2.4) (Figure 2.3b). The dry mass remaining in the cover crop catch frame treatment was slightly higher compared to the amended treatment with on ground harvest, although not significant. The AHS amendment and CC mix both declined relatively linearly over the 192- and 90-day time periods, respectively.

Table 2.4. After Fall 2022 AHS application, final dry average percent net mass remaining in AHS and CC litter bags in 2023 at given time points.

Treatment	C:N Ratio	Time Length (Days)	Total Water Applied (inches)	Avg. % Net Mass Remaining
Hull/Shell Mix	49:1	192	38.37	62%
Cover Crop Mix	18:1	90	16.16	31%

2.3.4 Soil aggregate stability

There were no significant differences in mean weight diameter between soil treatments from aggregate stability soil samples collected in Fall 2022 (Figure 2.4). However, in the small macroaggregate fraction, the amended and amended catch frame soil were significantly larger than the control. In the microaggregate fraction, the amended catch frame soil was significantly smaller than the amended and control soils (Table 2.5).

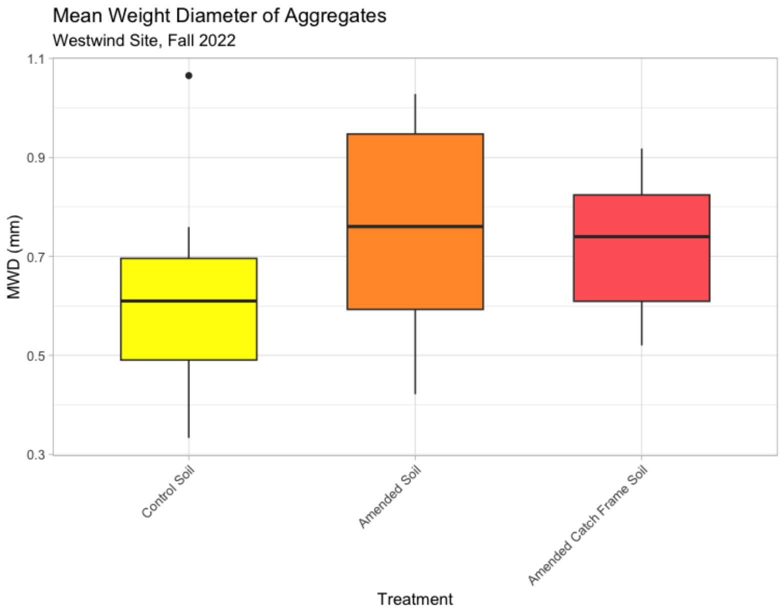
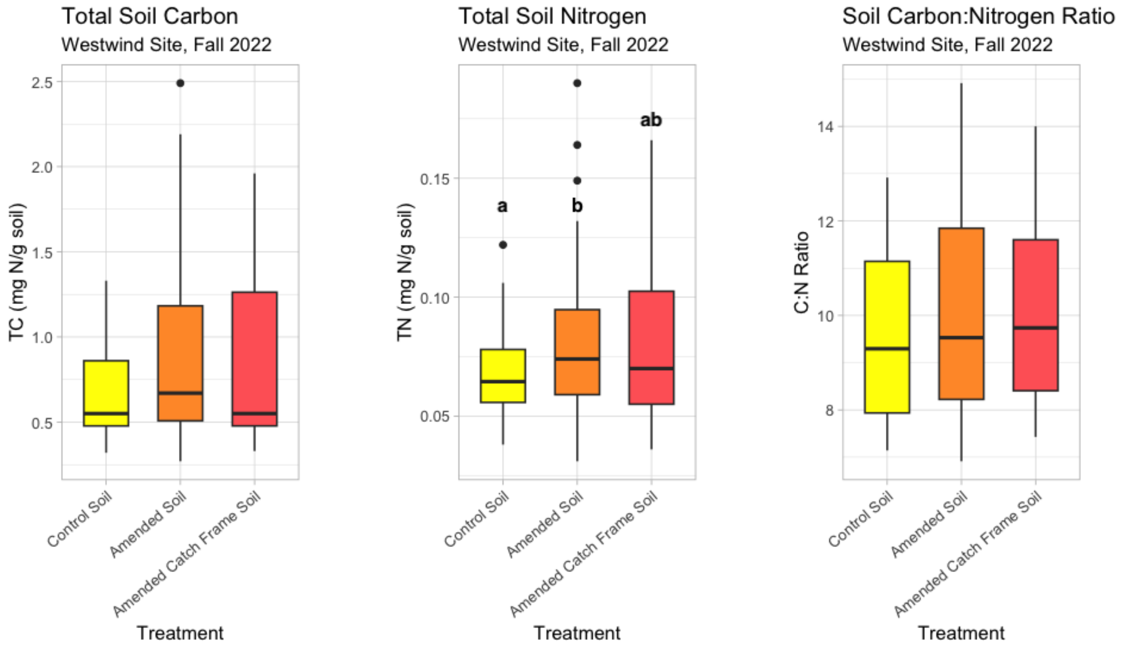


Figure 2.4. Mean weight diameter of soil aggregates sampled at a depth of 0-6 cm on 9/13/22 in Control Soil, Amended Soil, and Amended Catch Frame Soil. No significant differences between treatments.

Table 2.5. Distribution of macroaggregates and microaggregates sampled on 9/13/22 in control on-ground soil (T1), amended on-ground soil (T3), and amended catch frame soil (T4). Letters indicate significant differences between treatments within fraction size and sampling time point, using a threshold of p=0.05.

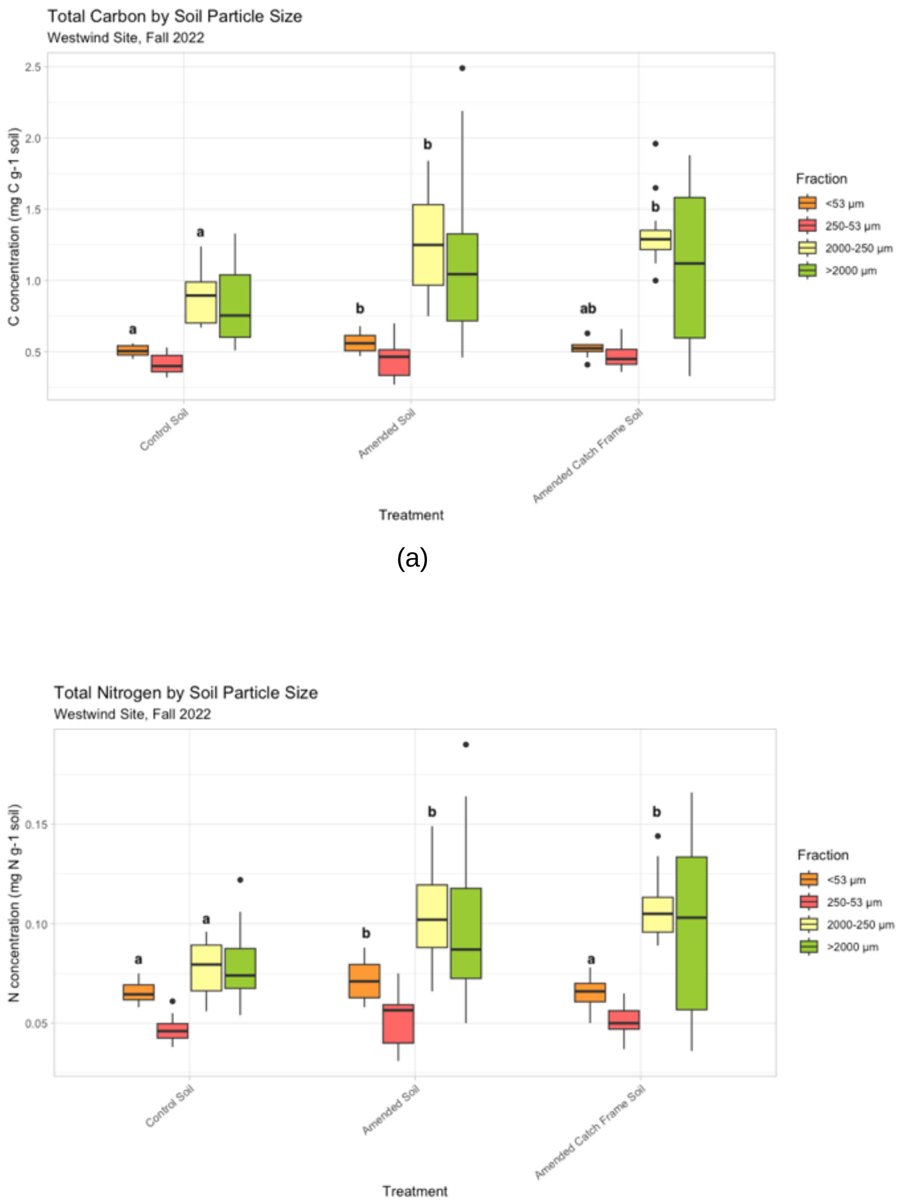
Treatment	Large macroaggregates >2mm (%)	Small macroaggregates 2mm-250µm (%)	Microaggregates 250µm-53µm (%)	Small microaggregates <53µm (%)
T1: Control Soil	17.0	18.0 a	38.6 a	26.4
T3: Amended Soil	21.0	25.7 b	32.0 a	21.3
T4: Amended Catch Frame Soil	18.2	26.6 b	31.7 b	23.4

There were no significant differences across soil treatments in Total Carbon (TC) or in C:N ratios, but there was significantly higher Total Nitrogen (TN) in the amended soil compared to the amended catch frame and control soil (Figure 2.5). However, when soils were separated across four fraction sizes, the TC, TN, and C:N ratio indicated largest contributions to the amended soil and amended catch frame soils from the large and small macroaggregate fractions (Figure 2.6, Table 2.6). TC was significantly higher in the amended catch frame and amended soil compared to the control in the small macroaggregate fraction (2mm-250µm). There was also significantly higher TC in the amended soil compared to the control in the small microaggregate fraction (<53µm), although there were no significant differences between amended catch frame and amended soils in the smallest fraction (Figure 2.6a). TN was also significantly higher in the amended catch frame and amended soil compared to the control in the small macroaggregate fraction (2mm-250µm). However, TN was significantly higher in the amended soil compared to the amended catch frame and control soils (Figure 2.6b). The C:N ratio was significantly higher in the amended catch frame and amended soils when compared to the control in the small macroaggregate fraction (2mm-250µm) (Table 2.6).



(a) (b) (c)

Figure 2.5a and b, and c. Total soil carbon, nitrogen, and C:N ratio sampled on 9/13/22 in Control Soil, Amended Soil, and Amended Catch Frame Soil. Letters indicate significant differences between treatments within fraction size, using a threshold of p=0.05.



(a)

(b)

Figure 2.6a and b. Total soil carbon and nitrogen sampled on 9/13/22 in Control Soil, Amended Soil, and Amended Catch Frame Soil and separated by fraction sizes. Letters indicate significant differences between treatments within fraction size and sampling time point, using a threshold of p=0.05.

Table 2.6. Average C:N ratio of macroaggregates and microaggregates sampled on 9/13/22 at Westwind in control on-ground soil (T1), amended on-ground soil (T3), and amended catch frame soil (T4). Letters indicate significant differences between treatments within fraction size and sampling time point, using a threshold of p=0.05.

Treatment	Large macroaggregates >2mm (%)	Small macroaggregates 2mm-250µm (%)	Microaggregates 250µm-53µm (%)	Small microaggregates <53µm (%)

T1: Control Soil	10.5	11.4 a	8.81	7.77
T3: Amended Soil	11.5	12.4 b	8.73	7.82
T4: Amended Catch Frame Soil	10.7	12.3 b	9.19	8.04

2.3.5 Soil organic matter fractions

Initial Fall 2022 soil data showed that % TC was significantly higher for mineral associated organic matter (MAOM) fractions in the amended catch frame and amended soils compared to the control. While % TC was significantly higher for particulate organic matter (POM) fractions in the amended catch frame and amended soils compared to the control, there were no significant differences between amended catch frame and amended soils (Table 2.7). Whereas %TN was significantly higher for MAOM fractions in the amended catch frame and amended soils compared to the control. There were no significant differences in % TN for POM fractions across all treatments (Table 2.7). The C:N ratio was significantly higher in the amended soil in the POM fraction compared to the control soil, however there were no significant differences between the amended and amened catch frame soils (Table 2.7).

Table 2.7. Average % Total C, %, and C:N ratio of POM (macroaggregates) and MAOM (microaggregate) fractions sampled on 9/13/22 at Westwind in control on-ground soil (T1), amended on-ground soil (T3), and amended catch frame soil (T4). Letters indicate significant differences between treatments within fraction size and sampling time point, using a threshold of p=0.05.

Fraction	Treatment	% TC	% TN	C:N Ratio
POM	T1: Control Soil	1.23 a	0.149	8.26 a
	T3: Amended Soil	1.56 b	0.179	8.64 b
	T4: Amended Catch Frame Soil	1.51 ab	0.177	8.52 ab
MAOM	T1: Control Soil	0.571 a	0.0450 a	12.5
	T3: Amended Soil	1.035 b	0.0761 b	13.3

	T4: Amended Catch Frame Soil	1.157 b	0.0845 b	13.6

2.3.6 Soil microbial community

After 8 months of AHS OMA application, soil samples were collected for phospholipid fatty acid analysis (PLFA). The amended catch frame soil had significantly higher average total biomass, total fungi biomass, saprophyte biomass, protozoa biomass, gram (+) biomass, undifferentiated biomass, and microbial diversity index compared to the control soil. The fungi:bacteria, gram (+):gram (-), and saturated:unsaturated, monounsaturated:polyunsaturated ratios were slightly higher in the amended catch frame soil compared to the control, although not significantly different. In the principal components analysis (PCA) biplot, increased gram-negative biomass and total bacteria biomass were the most distinct vectors that characterized the amended catch frame soil (Figure 2.7, Table 2.8). These vectors appeared to be correlated with actinomycetes biomass and gram-positive biomass, and less correlated with arbuscular mycorrhizal biomass, total biomass, and undifferentiated biomass.

The canonical correspondence analysis (CCA) plot showed that soil variables XK, XCa, pH, SOM, XMg, XNa, and CEC were changing along the axis towards the amended catch frame treatment which may indicate that microbial community composition was correlated to these variables, while less correlated with bulk density and nitrate (Figure 2.8). While nitrate indicated separation between amended and amended catch frame treatments, bulk density did not. Aggregate stability did not contribute significantly to the amended catch frame cluster, but the direction of the vector is trending towards that treatment, which may indicate potential microbial community shifts driving aggregate stability over time. There were no significant differences using an ANOVA with the CCA.

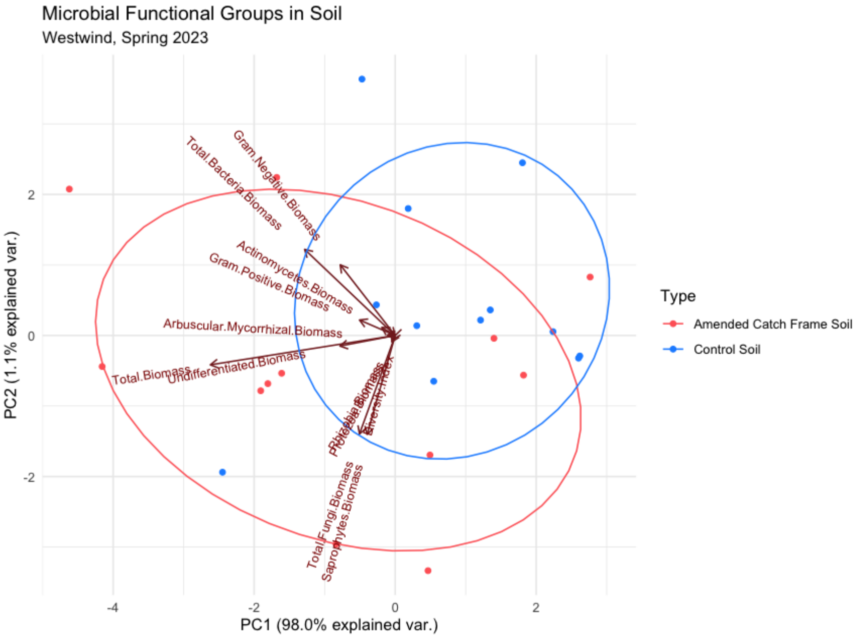


Figure 2.7. Principal components analysis biplot comparing microbial community composition in control and amended catch frame soils on 5/15/23.

Table 2.8. PLFA response variables from soil sampled on 5/22/23 at Westwind from the top 0-10 cm of the soil in control on-ground soil (T1) and amended catch frame soil (T4). Letters indicate significant differences between treatments within each response variable and sampling time point, using a threshold of p=0.05.

Response Variable	Microbial Biomass (ng g-1)			
	T1: Control Soil		T4: Amended Catch Frame Soil	
	Mean	Std. Dev.	Mean	Group
Total Biomass	2662 a	771.15	3523 b	1192.36
Diversity Index	1.35 a	0.04	1.43 b	0.09
Total Bacteria Biomass	1083	377.71	1462	607.62
Actinomycetes Biomass	173 a	47.71	233 a	106.30
Gram Negative Biomass	754 a	292.07	948 a	354.35
Rhizobia Biomass	0 a	0	17.8 a	34.25
Total Fungi Biomass	609 a	187.11	814 b	213.94
Arbuscular Mycorrhizal Biomass	95 a	34.36	131.5 a	61.98
Saprophytes Biomass	514 a	159.15	683 b	169.54
Protozoa Biomass	7.69 a	5.55	36.03 b	41.09
Gram Positive Biomass	329 a	94.02	515 b	273.76
Undifferentiated Biomass	961 a	225.96	1210 b	365.06
Fungi:Bacteria Ratio	0.584 a	0.11	0.595 a	0.12
Gram (+):Gram (-) Ratio	0.456 a	0.07	0.525 a	0.11
Saturated: Unsaturated Ratio	0.852 a	0.09	0.881 a	0.07
Monounsaturated: Polyunsaturated Ratio	2.66 a	0.78	2.87 a	1.08

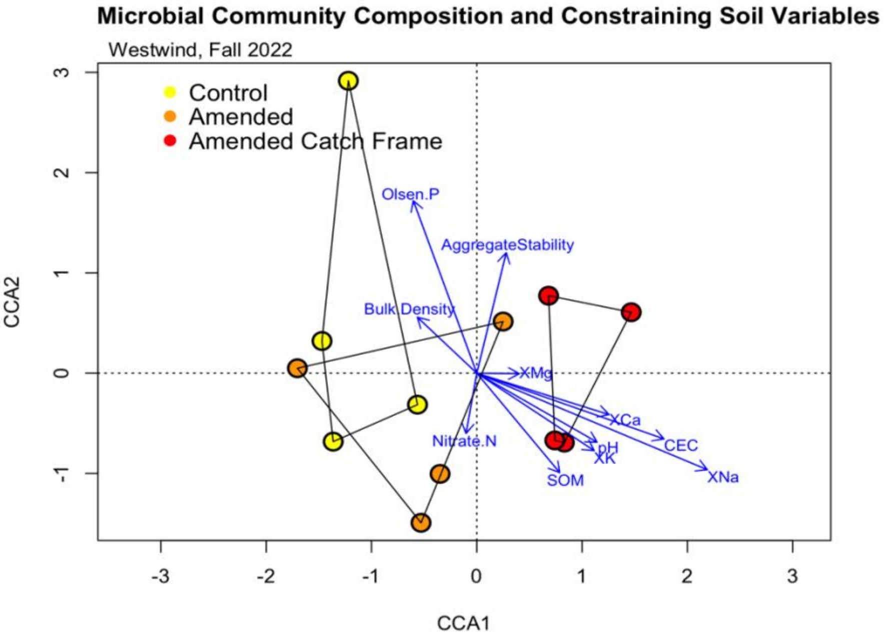


Figure 2.8. Canonical correspondence analysis plot with blue vectors displaying soil fertility and soil physical response variables in relation to microbial community biomass, represented by the clustered colored points for control, amended, and amended catch frame treatments sampled in Fall 2022.

2.3.7 Soil-water relations

Soil moisture, permittivity, soil conductivity, and PWEC increased, and soil temperature was moderated from May to August 2023 in the top 0-10 cm of soil (Figure 2.9). Combined and monthly average soil moisture, permittivity, and conductivity were significantly higher in the amended catch frame soil compared to the control. The monthly average differences were greatest during the month of June with a soil moisture difference of 2.9%, a soil permittivity difference of 2.4, and a soil conductivity difference of 280. Although monthly average soil temperature was not significantly different between the control and amended catch frame soil, the combined soil temperature across the months measured showed more moderated temperature during 10:00 until 14:00. During peak heat hours at 13:00 until the evening, the amended catch frame soil had lower overall soil temperature than the control which indicated that the amendment provided soil temperature relief. Combined monthly and hourly average soil conductivity was generally higher during early mornings before 06:00 and in the evenings in the amended catch frame soil. Combined monthly average soil PWEC was generally higher during wetter months and hourly average soil PWEC showed a steep decline approximately at 08:00, with a linear increase until 18:00, and finally a decrease towards the initial values in the evening. Combined monthly soil moisture and permittivity showed similar trends with the most significant difference in the month of June. Average hourly soil moisture and temperature showed the most significant differences during mid-day when the maximum air temperature was the hottest. In June the average maximum air temperature was 30.4 °C (86.7 °F). Additionally in July, the average maximum air temperature was 35.5 °C (95.9 °F) and the monthly average soil moisture was significantly higher in the amended catch frame soil.

From 5/3/2023 to 8/8/2023, the overall control soil was drier than the amended catch frame soil at a depth of 0-10 cm. The Acclima TDR probes indicated that the amendment increased soil moisture, moderated soil temperature, and increased soil permittivity, conductivity, and pore water electrical conductivity (PWEC). Average monthly soil moisture, temperature, permittivity, conductivity, and PWEC were higher in the amended catch frame soil compared to the control (Table 2.9). However, hourly average soil temperature appeared to have no differences during mid-day but indicated decreased soil temperature in the amended catch frame soil compared to the control as the day progressed (Figure 2.9).

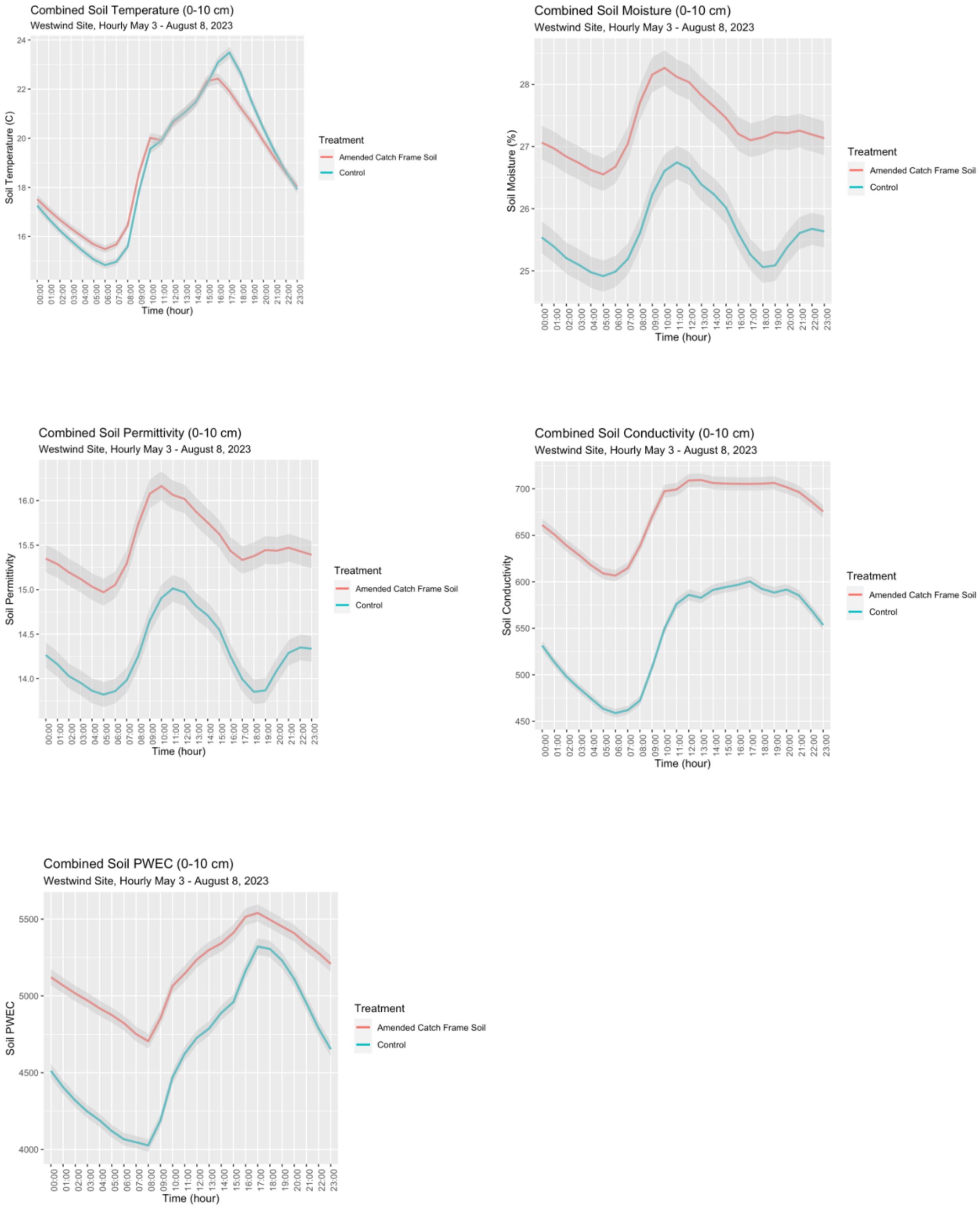


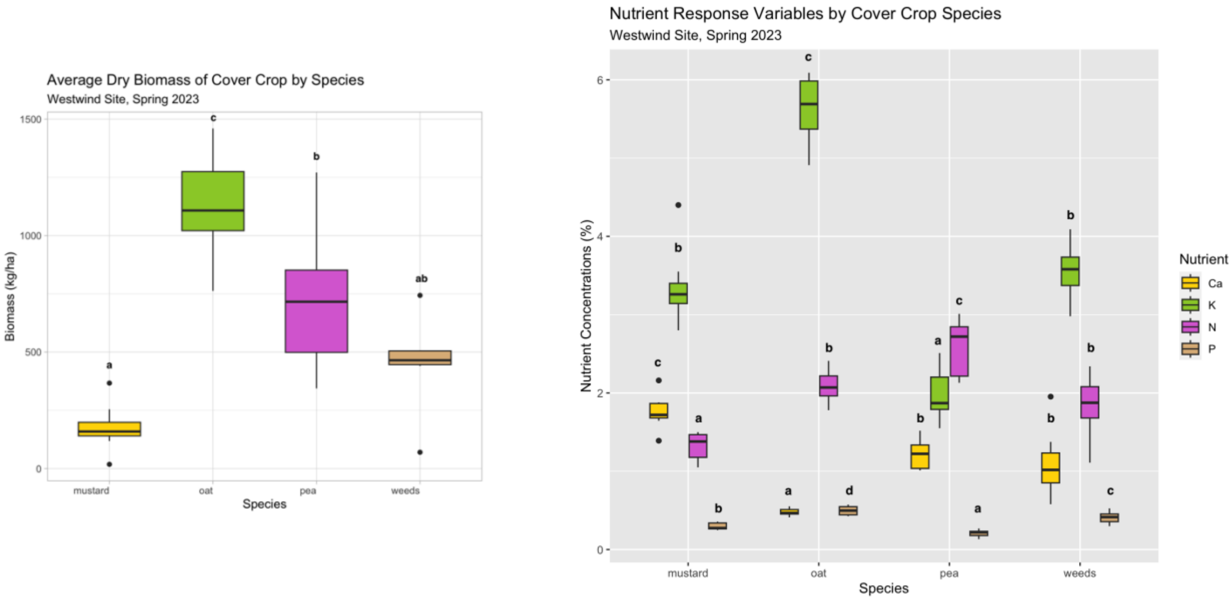
Figure 2.9. Hourly average soil moisture, temperature, permittivity, conductivity, and PWEC across all dates measured in the upper 0-10 cm (0-4 inches) soil with Acclima TDR probes.

Table 2.9. Monthly average soil moisture, temperature, permittivity, conductivity, and PWEC across all dates measured in the upper 0-10 cm (0-4 inches) soil with Acclima TDR probes at Westwind in the control on-ground soil (T1) and amended catch frame soil (T4). Letters indicate significant differences between treatments and sampling time point, using a threshold of p=0.05.

Month	Treatment	Moisture (%)	Temperature (℃)	Permittivity	Conductivity	PWEC
May 3-31	T1: Control Soil	32.1 a	11.5	18.5 a	437 a	2541
	T4: Amended Catch Frame Soil	33.1 b	11.7	19.5 b	459 b	2544
June 1-30	T1: Control Soil	27.5 a	20.4	15.4 a	685 a	5026 a
	T4: Amended Catch Frame Soil	30.4 b	21.4	17.6 b	965 b	6210 b
July 1-31	T1: Control Soil	20.8 a	19.8	11.2 a	405 a	4556
	T4: Amended Catch Frame Soil	23.3 b	20.0	12.7 b	538 b	4932
August 1-8	T1: Control Soil	22.2	23.0	12.1	644 a	6392 a
	T4: Amended Catch Frame Soil	22.4	23.4	12.2	729 b	6957 b

2.3.8 Cover crop biomass, nutrients, and soil fertility

After 5 months since the CC were seeded, average aboveground cover crop biomass separated by species was significantly higher in oat, followed by pea, and mustard. The dominant weed species was filaree, and its average biomass was slightly less than pea and slightly greater than mustard (Figure 2.10a). Plant nutrients N, P, K, and Ca were assessed across pea, oat, mustard, and weed species on 4/25/23 upon termination (Figure 2.10b). Across all species, % N in peas were significantly greatest, while %N in oats and weeds were significantly greater than mustard. The % P were statistically different across all species with the greatest value in oats, followed by weeds, mustard, and peas. Additionally, % K was significantly greatest in oats, while % K in in mustard and weeds were significantly greater than peas. Across all species, % Ca was significantly greatest, while % Ca was significantly greater in pea and weeds compared to oats. Average C:N ratio was significantly higher in mustard compared to oats, peas, and weeds (Figure 2.11). Average C:N ratio was significantly lower in peas compared to the other species. Although, average C:N ratio of oats were slightly higher than peas and weeds were slightly higher than oats, there were no significant differences. Soil samples were collected immediately after aboveground biomass was removed on 4/25/23 (Table 2.10). Nitrate-N was significantly higher in the control soil compared to the CC catch frame soil, while XMg was significantly higher in the CC catch frame soil compared to the control soil.



(a) (b)

Figure 2.10a and b. Average aboveground biomass and nutrient response variables by cover crop species sampled on 4/25/23. Letters indicate significant differences between species at sampling time point using a threshold of p=0.05.

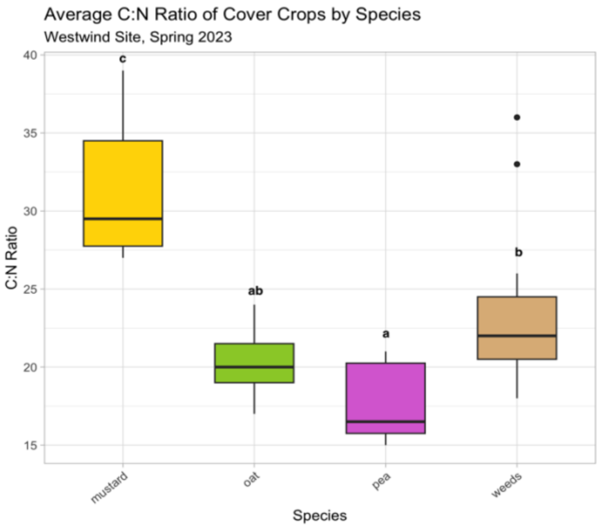


Figure 2.11. Average C:N ratio by cover crop species sampled on 4/25/23. Letters indicate significant differences between species at sampling time point using a threshold of p=0.05.

Table 2.10. Soil samples at 0-10 cm depths, were collected on 4/25/23 at Westwind following cover crop termination in control on-ground soil (T1) and cover crop catch frame soil (T2). Letters indicate significant differences between treatments within sampling time point, using a threshold of p=0.05.

Treatment	NO3-N (ppm)	Olsen-P (ppm)	XK (ppm)	XNa (ppm)	XCa (meq $100g^{-1}$)	XMg (meq $100g^{-1}$)	CEC (meq $100g^{-1}$)	% OM	pH
T1: Control Soil	5.29 a	2.25	115	21.0	15.4	3.02 a	18.8	2.27	7.14
T2: Cover Crop Catch Frame Soil	2.03 b	1.55	106	14.2	15.4	2.75 b	18.5	2.20	7.05

2.3.9 Yield and harvest equipment

During harvest in August 2023, yield in every individual tree row was collected with a weigh wagon for on-ground harvest treatments (T1 and T3), and a catch frame TOL harvester (Twin D T4 Shaker) for off-ground treatments (T2 and T4). Yield was impacted in the third year of applied AHS amendments. Average dry kernel yield was significantly higher in the amended treatment compared to the control, CC off-ground harvest, and amended off-ground harvest treatments. In contrast, average dry kernel yield was significantly lower in the CC off-ground treatment compared to the control, amended on-ground, and amended off-ground treatments. The average dry kernel yield was significantly lower in the amended off-ground treatment compared to the amended on-ground treatment, however, the control treatment was not significantly different compared to both the amended on and off-ground treatments. There were no significant differences in average dry kernel yield, average percent crack out, or average percent dry hull/shell trash in the yield samples. However, the average percent of total dry trash in the yield samples was significantly higher in the amended on-ground treatment compared to the off-ground treatment (Table 2.11). In summary, average total trash and average kernel yield was highest in the amended on-ground treatment.

Table 2.11. Average yield data was collected on 8/14/23 at Westwind in the control on-ground treatment (T1), cover crop off-ground treatment (T2), amended on-ground treatment (T3), and amended off-ground treatment (T4). Letters indicate significant differences between treatments within sampling time point, using a threshold of p=0.05.

Treatment	Avg. Dry Kernel (lb/ac)	Std. Dev. (lb/ac)	Avg. % Crack Out	Avg. Dry HS Trash in Yield Samples (%)	Avg. Total Dry Trash of Yield Samples (%)
T1: Control On-Ground	2238 bc	158	27.5%	4.02%	12.7% a
T2: Cover Crop Off-Ground	1752 a	305	27.3%	4.34%	11.3% a
T3: Amended On-Ground	2421 c	554	27.4%	2.54%	23.2% b
T4: Amended Off-Ground	2100 b	229	27.4%	2.46%	4.58% a

2.4 Discussion

2.4.1 Almond hull and shell nutrients and decomposition

After the Fall 2022 AHS application, the average C:N ratio of the AHS were 64:1 and decreased over time with a ratio of 49:1 in Spring 2023. We expected lower C and N values during the 192-day period from the initial AHS litter bag installation to the final collection. We found that % C decreased, but % N significantly increased in the Spring. Although other experiments have found that lower quality litter with high C:N ratios caused N immobilization in tropical forests where litter is continuous and undisturbed (Andrews et al., 2021; Leal et al., 2023), our experiment utilized precise treatments applied in a field trial and thus, the increased N in the amendment layer may have resulted from compost applied and fertigation events throughout the season. This may suggest that N microbial biomass in the amendment was retained since 62% of the net mass remained from 10/17/22 to 4/25/23.

Decomposition may also have slowed because the overall AHS mix applied in the Fall consisted of only 25% hulls and 75% shells. The largest nutrient source remains in the almond hulls (Valverde et al., 2013), while our predominate mixture contained mostly shells, which may explain the slight decrease in % C over time and slower decomposition process. In the AHS mix, Total C showed the most similar decline as the decomposition rate since Total N was maintained over time. The parallel decomposition rates of the overall AHS mix and Total C, suggests that the amendment layer provided a suitable environment for microbial functionality and decomposition. In the AHS mix, % K significantly decreased from 1.89% to 0.107% from Fall 2022 to Spring 2023. Previous studies have found that plant materials such as cacao husks, maize, and pecan shells rapidly solubilize K and with the addition of water and rainfall, K is released even more rapidly (Andrews et al., 2021; Idowu et al., 2017). Continued yearly AHS applications can provide a source of readily available K for almond trees.

2.4.2 Soil fertility and soil-water relations

XK was the only soil fertility variable that was significantly higher in the amended treatments compared to the two unamended treatments and indicated similar trends across depths of 0-10 cm, 10-20 cm, and 20-30 cm. Interestingly, soil pH was significantly higher in the unamended off-ground treatment compared to the amended on-ground treatment and was slightly greater than both the control and amended off-ground treatments. A long-term study in an avocado orchard compared conventional and organic treatments using almond shells and found that the amendment significantly reduced soil pH to neutral compared to the control (López et al., 2014). Researchers also discovered that increased Kjeldahl-N and Olsen-P resulted from the reduced soil pH, which suggested that the amendment was able to mobilize unavailable soil P. However, in our field experiment, Olsen-P was slightly lower in both amended treatments, although not significant. However, other studies have found that pH increased with the addition of crop residues in two soil column experiments that utilized canola and chickpea amendments (Butterly et al., 2013; Wang et al., 2017). It was found that the chickpea amendment increased pH from 6.2 to 7.5 from Butterly et al., 2013 and from 4.5 to 4.97 from Wang et al., 2017. Although there are various context specific conclusions about the impacts of crop residues increasing or decreasing soil pH, one explanation for increased pH may be a result of excess cations in organic amendments. In our study, CEC at 0-10 cm was slightly higher in the amended off-ground treatment compared to the other three treatments, which may suggest that maintaining the amendment layer can potentially improve CEC and moderate soil pH. Our baseline soil samples showed that % OM was not significantly different across all four treatments. However, it is worth noting that % OM was slightly higher in the amended treatments and had higher values at depth 0-10 cm and declined with deeper depths.

The relationship between soil texture and water infiltration was demonstrated in an experiment utilizing ground pecan husks, ground pecan shells, and ground pecan shell biochar. The researchers determined that soil moisture increased in the sandy loam and clay loam soils for pecan hulls and pecan shell biochar treatments, but not in the sandy clay soil (Idowu et al., 2017). They also found that evaporation decreased but that it varied based on soil texture. Short term soil moisture increased under all three amendments in the sandy loam and clay loam soils but there were no effects on the sandy clay loam soil. However, the soil moisture content decreased in the pecan husk treatment in sandy soils which is likely due to poor water retention in fine textured soils. Other studies have found that organic mulches that included straw mulch (Chen et al., 2007), rice husks (Iqbal et al., 2020; Linam et al., 2023) and hazelnut husks (Mennan & Ngouajio, 2012), reduced overall soil temperature by providing a barrier between the air temperature and the soil. In an experiment that used straw mulch, researchers found that during warmer months, the mulch was able to reduce the average soil temperature compared to the control in a winter wheat field, however the lower temperature reduced the early growth period needed for biomass development (Chen et al., 2007). The rice mulch improved soil moisture over time and demonstrated that applying mulch at optimal times for different cropping systems is imperative for yield. Future research is needed to study the implications of measuring soil water dynamics at different depths, soil types, climates, and irrigation management practices. Our study did not grind the AHS amendment before application, but long-term studies are needed to assess the decomposition of ground materials and the implications of the overall tree status from crop residues.

2.4.3 Physiochemical properties

In Fall 2022, there were no significant differences in MWD of soil aggregates between the control, amended, and amended catch frame soils. However, the percentage of soil in the small macroaggregate fraction (2mm-250µm) was significantly higher in both the amended and amended catch frame soils compared to the control soil. Similarly, the percentage of the soil in the microaggregate fraction (250µm-53µm), was significantly higher in the amended catch frame soil compared to the amended and control soils. In an experiment that studied the effects of irrigation and maize litter on SOM, researchers found that the average C:N ratio was highest in the macroaggregate fraction followed by variable ratios in the microaggregate fraction, with lowest values in the MAOM fraction (Núñez et al., 2022). Overall, total soil N was highest in the amended and amended catch frame soils compared to the control, but there were no significant differences in total soil C and the C:N ratio across all treatments. However, when separated by fraction size, the average C:N ratio was significantly higher in both the amended and amended catch frame soils compared to the control in the small macroaggregate fraction (2mm-250µ). Although most field experiments studying the effects of crop residues on SOM average 10 years (Franzluebbers et al., 2000; Sayer et al., 2021; Xiao et al., 2022), our results supported their findings and may suggest that our 2-year amendment has improved soil aggregation from increased C and N in the amended soils likely a result of increased microbial activity.

We hypothesized that the previous surface applied AHS OMA would increase soil C in the >53µm POM macroaggregate fraction compared to the <53µm MAOM microaggregate fraction. Our results showed overall % TC was highest in POM compared to MAOM and was significantly higher in the amended and amended catch frame soils compared to the control. MAOM also showed similar trends with significantly higher % TC in the amended and amended catch frame soils compared to the control. In a 13-year experiment that studied C and N in soil particle size fractions from altered litter inputs in a lowland tropical forest, researchers found that the largest amount of C and N was in the MAOM fraction and smallest in the POM fraction. Since POM is controlled by microbial and enzymatic inhibition, such as freezing temperatures or waterlogging from amendment applications, POM may be more sensitive to SOM formation (Lavallee et al., 2020; Lehmann & Kleber, 2015). Although our application rate for AHS OMA was high and could potentially create anoxic soil conditions, we did not find that to be the case (Andrews et al., 2023). In fact, our results showed that the increased % TC in POM may contribute to the formation of MAOM for overall SOM stabilization and C storage (Angst et al., 2023). The microaggregate fraction (MAOM) is an important indicator of soil stabilization and our findings showed that there were higher percentages of soil in both the micro and macroaggregates fractions, which aligned with other studies that found increased mycorrhizal fungal hyphae, microbial biomass, and C stabilization formed from stable microaggregates (Franzluebbers et al., 2000; Moreno-Ramón et al., 2014; Rieke et al., 2022; Six et al., 2000).

The overall C:N ratio and % TN was highest in MAOM but the C:N ratios were significantly higher in the amended and amended catch frame soils in POM. MAOM is abundant in N compared to initial plant materials and the degradation of MOAM can supply N required for litter decomposition, as demonstrated in our results. Since C is strongly linked to N availability that is needed to decompose crop residues into stable SOM additional research is needed to study the negative impacts that may affect the microbial degradation process of MAOM formation from excess N via fertilization or amendments (Sayer et al., 2021). There were no significant differences in C:N ratio across all three treatments in the MAOM fraction, but other studies have found that low C:N ratios in MAOM can contribute large sources of N to the rhizosphere (Lavallee et al., 2020; Sayer et al., 2021). POM that has not undergone significant decomposition, will have approximately the same C:N ratio as the initial plant material. Our data suggested that the AHS OMA had an initially high C:N ratio that decreased drastically over time which implied that C and

N sources were being utilized by microbes. Although our use of a lower quality amendment with an initial high C:N ratio may favor the formation of POM over MAOM, more research needs to be done to better understand the possibilities for POM to be sequestered in the long term.

2.4.4 Soil microbial community functions

In Spring 2023, the amended catch frame soil had significantly higher average total biomass, total fungi biomass, saprophyte biomass, protozoa biomass, gram (+) biomass, undifferentiated biomass, and microbial diversity index compared to the control soil. We expected the fungi:bacteria ratio to be higher in the amended catch frame soil compared to the control due to increased arbuscular mycorrhizal fungi and saprophyte biomass (Chowdhury et al., 2022), which was confirmed by our results. The fungi:bacteria, gram (+):gram (-), saturated:unsaturated, and monounsaturated:polyunsaturated ratios were slightly higher in the amended catch frame soil compared to the control, although not significant. As seen in our results, these increased ratios may indicate that the increased formation of organic matter in the amended soil has caused simple C compounds to shift to more complex C compounds which is commonly found in ecosystems dominated by fungi (Bolan et al., 2011; Jackson et al., 2017).

Increased decomposers in the amended catch frame soil from saprophytes and actinomycetes likely contributed to the decay of C compounds in the AHS material. Saprophytes aid in the degradation of chitin and lignin and facilitate SOC turnover by increasing microbial C oxidative activity, which is a crucial process when decomposing organic amendments with high C:N ratios. Overall, total bacteria, gram (+) bacteria, and gram (-) bacteria all increased as shown in our PCA plot in the results section. Saprophytic communities tend to increase with decreased soil fertility, whereas bacteria rich soils are usually abundant with high SOC (You et al., 2014). In soils with a high clay content, bacterial biomass tends to increase while saprophytic fungal biomass decreases.

Our field site was a sandy clay San Ysidro soil with approximately 35% clay (SoilWeb, 2023), which seemed to support a diversity of bacteria and fungi.

Protozoa biomass was approximately fivefold greater in the amended catch frame soil compared to the control, which indicated that the amendment layer could support high tropic levels in the soil food web. The primary roles of protozoa are to accelerate the decomposition of microbial biomass, contribute nutrient mineralization (N mineralization), and to regulate the composition of the microbial community (Hodson et al., 2021). Protozoa also form interactions with roots to excrete nutrients for further microbial growth and would be beneficial in amended or cover cropped soils where microbes return nutrients back to the soil, potentially increase nutrients for main crops, and proliferate as a result of this beneficial mutualism (Bowles et al., 2015; Griffiths, 1994; Kästner et al., 2021; Wu et al., 2023). Future research can assess microbial communities across AHS OMA and various CC species to identify specific microbe functions from targeted ecological management strategies.

2.4.5 Cover crop nutrients, decomposition, biomass, and soil fertility

A CC mix of 60% spring pea, 35% oat, and 5% yellow mustard was selected to include a diverse species mix that would produce a high biomass in the grass species, provide soil mineral N from the peas, and attract pollinators from the mustard (Bugg & Waddington, 1994; Devi, 2021; Florence et al., 2019; Koudahe et al., 2022). At CC termination in Spring 2023, average aboveground biomass was significantly higher in oat, followed by pea, and mustard. The dominant weed species was filaree, and its average biomass was slightly less than pea but slightly greater than mustard. The % N in peas were significantly greatest compared to all species, while % N in oats and weeds were significantly higher than mustard. Both the % P and % K were also significantly higher in oats compared to the other species.

Approximately 69% of the CC net mass remained from 4/26/23 to 7/26/23 (90 days), which suggested that the CC material was highly decomposed, however the net dry mass remaining was slightly higher in the cover crop treatment compared to the amended treatment, although not significant. Our data may suggest that the amendment did not affect the decomposition process of the CC material since there were no significant differences between treatments. However, other CC and mulch experiments in orchards have conflicting results that showed increased weed control with surface applied nutshell and husk mulch, whereas others have shown neutral responses to CC growth with applied mulch materials (Iqbal et al., 2020; Mennan & Ngouajio, 2012; Webber et al., 2022). We expected the CC material to be decomposed more quickly than the AHS material since the initial average C:N ratio was 23:1 compared to 64:1 in the AHS material. At the final timepoint, approximately 16.16 inches of water from irrigation were applied, which aided overall decomposition.

XMg was significantly higher in the CC catch frame soil compared to the control soil, while Nitrate-N was significantly higher in the control soil compared to the CC catch frame soil. Previous research at our site found that the high inputs of K from the AHS may displace soil cations such as Na and Mg (Andrews et al., 2023), thus including another crop residue from a CC may replace the Mg lost as other studies have found increase available Mg from mulches (Merwin et al., 1995; Salau et al., 1992).

We initially expected higher soil N in the CC treated soil, however a study that evaluated CC as buffer strips to manage soil nitrate found that a 6m and 9m buffer strip seeded with perennial ryegrass reduced nitrate by 42% and 46%, respectively (Novara et al., 2013). This may suggest that CC are able to absorb excess soil nitrate, thus resulting in lower soil Nitrate-N levels as demonstrated in our results. Another experiment found that a vetch legume CC treatment increased soil nitrate the most compared to the other treatments seeded with barley a mixture of barley and vetch but found that the CC improved SOM and SOC (Repullo-Ruibérriz de Torres et al., 2021). In another CC experiment, vetch and clover litter bags released N within 4 weeks, while N release diminished at week 10 (Kramer et al., 2002). CC may serve as an effective sink that in turn can stabilize increased N inputs and reduce N leaching (Kremer & Kussman, 2011). Other studies have found that crop residues resulted in lower soil N compared to the controls, likely due to organic mulches utilizing available N while in the growth stage (Boetzl et al., 2023; Broschat, 2007). Our data showed significantly higher aboveground biomass in oats compared to peas which may suggest that N uptake was occurring rather than being returned to the soil. Other studies have found that CC with a lower C:N ratio between 20 to 30, will decompose quickly and release N rapidly after termination because N immobilization is less of an issue compared to CC that have higher C:N ratios (Bugg et al., 1991; Rodriguez Ramos et al., 2022). We only sampled the soil below the grown CC at one timepoint, but further researcher is needed to study the release rates of N after CC termination and to investigate the effects of a legume CC crop mix for future N fertilizer management strategies in orchards.

2.4.6 Yield and harvest equipment

Yield was significantly higher in the amended on-ground treatment and slightly greater compared to the control on-ground treatment. The cover crop off-ground treatment was significantly lower compared to the other three treatments, likely a result of nutrients being allocated away from the trees and directed towards the CC growth. Other studies have reported lower or insignificant differences across orchard yield where CC were grown (Devi, 2021; Repullo-Ruibérriz de Torres et al., 2021). One experiment found no significant differences across almond yield treated with vetch, barley, a mixture of vetch and barley, and a control (Repullo-

Ruibérriz de Torres et al., 2021). Although final yields were slightly greater in all CC treated plots compared to the control, researchers observed slight improvements in soil-nitrate in the top 0-5cm of soil in the vetch treatment in 2016, and improvements in soil-nitrate again in 2017 in the vetch and legume mixture treatments. It was also found in another paper that in a medium to high density apple orchard where CC were planted, yield and tree development were stagnated (Devi, 2021). Although our mature almond field trial was not densely planted, fruit development may have been delayed due to competition of nutrients and water from the scavenger oat species in our CC mix. However, another paper studied the effects of reduced tillage, reduced tillage plus green manure, and no tillage on almond yield and soil characteristics. The green manure used consisted of a cover crop mix of common vetch and common oat that incorporated into the soil after Spring termination. Yield across a 10-year period showed that the reduced tillage management practice had the highest overall yield, but that differences between reduced tillage and reduced tillage plus green manure decreased over time, whereas the differences between reduced tillage and no tillage increased over time. This may have suggested that the addition of adding a cover crop to reduced tillage does not impact yield as much as the type of soil management practice. One benefit from the CC showed higher belowground biomass and significantly higher root length values in the reduced tillage plus green manure treatment compared to the reduced tillage only.

Although, we expected the amended off-ground treatment to have the highest yield because the catch frame harvest equipment would maintain the amendment on the ground, improve soil moisture, moderate soil temperature, and improve aggregate stability, interestingly, the amended off-ground treatment was significantly lower than the amended on-ground treatment. Previous studies have shown that mulching can improve root development, improve soil water content, and maintain optimal soil temperatures (Andrews & Kassama, 2022; Chalker-Scott, 2007; Iqbal et al., 2020; Webber et al., 2022). Our experiment did not study belowground biomass or root growth, however Andrews et al., 2023 found increased root biomass in the amended treatments which may contribute to microbial shifts in the soil with continued use of the amendment and off-ground harvest equipment. Therefore, further research is needed to better understand yield in the context of using on and off-ground harvest equipment and its effects on soil water relations, microbial communities, and soil compaction.

2.5 Conclusion

Almond hulls and shells used as organic matter amendments simulate forest litter ecosystems and can improve soil aggregate stability, increase microbial diversity, increase soil organic matter stabilization, and improve soil-water dynamics. To answer our question about how the effects of soil physical and biological properties under AHS OMA shifted with on and off-ground harvest after 3 years, we found that soil aggregation improved while microbial diversity increased over time. The use of off-ground harvest machinery has the potential to improve mulching effects, increase soil moisture, and improve soil structure which is vital for farmers who rely on soil functionality to grow almonds in a more sustainable way. Future research should consider how off-ground harvest effects yield while also measuring additional soil health benefits including improving aggregate stability, organic matter, and microbial community functions. To answer our second question about how Fall applied AHS OMA impact CC biomass, nutrient status, and short-term composition, we found that overall decomposition was rapid and that the CC acted as a buffer from soil nitrate. Further research can investigate different species of CC mixes and interactions with AHS OMA as potential strategies to manage nutrient inputs for overall water savings and soil benefits.

Supplementary

Supplementary Table 3.1. Fall baseline soil samples at depths 0-10 cm, 10-20 cm, and 20-30 cm were collected on 9/13/22 at Westwind in the control on-ground soil (T1), unamended catch frame soil (T2), amended on-ground soil (T3), and amended catch frame soil (T4). Letters indicate significant differences between treatments within depth and sampling time point, using a threshold of p=0.05.

Depth (cm)	Treatment	NO3-N (ppm)	Olsen-P (ppm)	XK (ppm)	XNa (ppm)	XCa (meq $100g^{-1}$)	XMg (meq $100g^{-1}$)	CEC (meq $100g^{-1}$)	%OM	pH
0-10	T1: Control On-Ground Soil	5.06	4.15	168 ab	1100	10.4	3.66	19.2 a	2.23	8.06
	T2: Unamended Catch Frame Soil	2.80	3.55	151 a	1088	12.3	3.69	21.1 ab	2.36	8.17
	T3: Amended On-Ground Soil	3.37	3.52	242 bc	1354	10.7	3.81	21.1 ab	2.38	7.93
	T4: Amended Catch Frame Soil	3.31	3.05	248 c	1387	12.3	4.02	23.1 a	2.40	8.04
10-20	T1: Control On-Ground Soil	1.50	1.40	110 ab	1563	11.5	4.12	22.8	1.60	7.79 ab
	T2: Unamended Catch Frame Soil	1.57	3.63	106 a	1466	12.4	4.29	23.4	1.73	7.91 b
	T3: Amended On-Ground Soil	1.09	2.08	194 b	1466	11.1	4.33	22.4	1.88	7.51 a
	T4: Amended Catch Frame Soil	1.29	1.93	182 ab	1308	12.6	4.40	23.2	1.80	7.83 ab
20-30	T1: Control On-Ground Soil	0.190	<1.0	97.2 a	1404	11.8	4.55	22.8	1.52	7.65 ab

	T2: Unamended Catch Frame Soil	0.333	3.00	91.5 a	1451	13.0	5.53	25.1	1.51	7.74 b
	T3: Amended On-Ground Soil	0.677	1.93	165.5 b	1275	11.8	4.56	22.3	1.75	7.43 a
	T4: Amended Catch Frame Soil	0.237	1.73	117.5 ab	1069	12.7	5.19	22.8	1.71	7.68 ab

Supplementary Table 3.2. CIMIS average monthly water inches from May to August 2023. 38.37 inches of irrigation water were applied in this 4-month period which released K quickly.

Month	Total ETo (inches)	Total Precipitation (inches)	Avg Max Air Temp (°F)	Avg Min Air Temp (°F)	Avg Air Temp (°F)
May	6.22	0.82	79.0	50.0	63.9
June	7.50	0.00	86.7	53.9	69.9
July	8.49	0.00	95.9	58.1	76.6
August	7.17	0.59	93.7	60.5	76.5

Supplementary Table 3.3. Explanations of Phospholipid Fatty Acid analysis response variables. Written by Ellie Andrews (UC Davis) using Ward Laboratories, Inc. (Kearney, NE) reports, 2023.

Response Variable	Significance and Functions
Functional group diversity index	Does treatment increase or decrease diversity? Indicates a broad/narrow range of microbe traits that influence functioning. The more diverse the carbon sources provided by the treatment, the more likely to increase DI.
Total microbial biomass	Does the treatment create conditions and resources that lead to more microbes? Indicates to what degree soil can support microbial life and biomass production. Treatments that supply carbon (and nitrogen) are more likely to increase TMB.
Fungi:bacteria ratio	Bacteria tend to dominate systems with lower organic residues, dry conditions, or after soil disturbances. Fungal-dominated communities tend to be more resilient to environmental stressors. Fungi tend to be considered good soil health indicators. Lower disturbance and increased organic residues tend to promote fungi.
Gram (+):gram (-) ratio	Higher gram (+) levels are common when the bacterial community is stressed or coming out of dormancy. Since they can form spores, they survive better under environmental stressors such as drought or extreme temperatures.

	Higher gram (-) levels may be due to anaerobic conditions or other stressors. The soil bacterial community tends to become more balanced (1.0-2.0 ratio) as soil conditions become more favorable during the growing season. Gram (+) have many-layered thick cell walls, while gram (-) have thinner cell walls. This ratio can help indicate relative carbon availability for soil bacteria: gram (-) are more dependent on simple C compounds from plants, while gram(+) are more dependent on complex C compounds in organic soils.
Actinomycetes (bacteria)	Gram(+), cycle organic matter and decompose complex mixtures of polymers such as cellulose and hemicellulose. They resemble fungi because they have long branching filaments (smaller than fungi). Some can fix nitrogen on legumes.
Rhizobia (bacteria)	Gram(-), form root nodules on legumes and fix nitrogen.
Arbuscular mycorrhizae (fungi)	Plant symbiont that enhances nutrient and water uptake. Improves plant stress tolerance.
Saprophytes (fungi)	Decomposers that drive nutrient cycling, availability, and CO2 flux. They facilitate SOC turnover by increasing microbial C oxidative activity. They aid in the degradation of chitin and lignin that can deconstruct complex C compounds and transfer nutrients through hyphae (Cowther et al. 2012, You et al., 2014).
Protozoa	Presence of protozoa indicate sufficient base level nutrients to support higher trophic levels beyond bacteria.
Undifferentiated	The vast majority of soil microbes still await identification.
Saturated : unsaturated	Reflects how bacteria may be altering their membranes under environmental stressors to maintain optimal fluidity and waste transport, so higher saturated fatty acids may indicate a more well-adapted community to present environmental conditions (temperature and moisture). A higher ratio means a healthier and more stable bacterial community.
Monounsaturated : polyunsaturated	Higher ratio means less stress. Lower ratio indicates higher levels of prolonged stress due to conditions such as temperature, moisture, pH, or nutrient availability (starvation).

Supplementary Table 3.4. Average cover crop nutrient concentrations sampled on 4/26/23 until 7/26/23. Letters indicate significant differences between each nutrient and sampling time point, using a threshold of p=0.05.

Nutrient	Initial Time: 4/26/23	Final Time: 7/26/23
N (%)	1.93 a	2.24 b
P (%)	0.365 a	0.472 b
K (%)	3.62	4.01
S (ppm)	5804	6256
B (ppm)	26.8	28.1
Ca (%)	1.118	0.974

Mg (%)	0.214 a	0.268 b
Zn (ppm)	66.8	71.6
Mn (ppm)	89.4 a	162.5 b
Fe (ppm)	590 a	1608 b
Cu (ppm)	7.41 a	8.57 b
C (%)	42.0 b	40.8 a
C:N Ratio	23.2 b	18.3 a

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