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Microbiome Response to Organic Matter Amendments in Almond Agroecosystems

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Abstract

The use of organic matter amendments (OMAs) is an ancient practice for improving agricultural soils, but our understanding of the microbial mechanisms by which benefits to soil ecosystems occur remains incomplete. The 1.6 million acres of almond orchards in California need effective and sustainable management practices that promote soil as a living ecosystem. This thesis examines OMAs in the context of emerging microbiome science, our current understanding of relevant soil processes as affected by OMAs, and the challenges involved in modeling nutrient cycling through the microbiome. Results from two research trials conducted in 2019-2021 to evaluate three soil health practices in almond agroecosystems are presented. First, a field trial explored the effects of an almond hull and shell amendment and off-ground harvest on soil C and N and soil microbial biomass in almond orchards. A 210-day incubation trial further evaluated the same amendment on soils with differing management histories by utilizing soil that had previously received a green waste compost OMA for three years. Results highlight the suitability of the almond hull and shell amendment as a soil health treatment, finding that it increased microbial respiration and microbial biomass without significantly affecting nitrogen immobilization. Soil with a history of past OMA application displayed higher microbial respiration, dissolved organic carbon, and net N mineralization than soil without that history when receiving a new amendment of almond hulls and shells. Decomposition of amendment residue progressed at nearly identical rates in the field trial and the incubation trial and exhibited characteristics comparable to forest litter layers. These findings add to our understanding of the processes by which OMAs impact the soil microbiome and our ability to utilize these processes to achieve specific outcomes.

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Chapter 1: Microbiome Response to Organic Matter Amendments

Introduction

Recent papers have argued that manipulating, cultivating, or otherwise impacting plant and soil microbiomes will soon be an important tool in agriculture (Fierer, 2017; French et al., 2021; Kehe et al., 2019; Mercado-Blanco et al., 2018; Toju et al., 2018; Volpiano et al., 2022). The extreme complexity of these systems, however, remains a barrier (French et al., 2021; Kehe et al., 2019; Toju et al., 2018). While the use of microbial products in agriculture is becoming more common (Bhattacharyya & Jha, 2012; French et al., 2021; Sessitsch et al., 2019), some question whether such an approach can achieve the desired results (Bacilio et al., 2017; French et al., 2021). Breakthroughs in methodology and in the analysis and interpretation of large data sets will be needed, particularly in the fields of genetics and bioinformatics (Barrios, 2007; French et al., 2021; Mercado-Blanco et al., 2018; Volpiano et al., 2022). At the same time, however, there are still open questions about microbial functions and processes in agroecosystems and how microbes respond to existing management techniques. Organic matter amendments (OMAs), for example, are an ancient practice in which organic materials, usually waste products, are returned to the soil ecosystem through microbial and microfaunal decomposition. Yet the precise effects of adding a given organic matter amendment to a given soil are still difficult to predict (Bonilla et al., 2012; Geisseler et al., 2021). Gaining a better understanding of the impact of common management practices on soil microbial communities and processes is necessary if microbiome manipulation is to become a useful tool. This review will summarize what we know about soil microbial response to organic matter amendments with a focus on orchard agroecosystems.

OMAs and the Importance of the Soil Microbiome

The effectiveness of OMAs for improving agricultural soils has been recognized for at least 2,300 years (Montgomery, 2012). OMAs represent a significant food source for microbial decomposers and add resources to the soil ecosystem. Microorganisms regulate or drive a vast number of ecological processes in the soil, including the cycling of plant nutrients such as carbon, nitrogen, phosphorus, iron, calcium, magnesium, manganese, and zinc (Fierer, 2017; Li et al., 2019; Volpiano et al., 2022). Microbial population growth and turnover is the basis of soil organic matter (SOM) formation and OMAs fuel this growth (S. L. Jansson & Persson, 1982). Previous theories on soil organic matter held that it was made up of recalcitrant plant material that was chemically or physically resistant to decomposition (Kleber, 2010; Lützow et al., 2006), but recent research has revealed that the majority of SOM is actually made up of dead microbial bodies (Liang et al., 2019; Miltner et al., 2009) or microbially-derived compounds (Cotrufo et al., 2013; Grandy & Neff, 2008; Lützow et al., 2006). A robust microbiome, therefore, is essential for SOM formation and global carbon storage and sequestration, which has garnered significant interest as a strategy for reducing atmospheric carbon (Lal, 2004).

Increased soil organic carbon (SOC) is correlated with desirable soil characteristics, and 90% of the decomposition of carbon in the soil is carried out by microorganisms (Barrios, 2007). Because of their desirable effects on soil agricultural performance, characteristics such as SOC have become metrics for assessing soil health. Soil health is defined by the Natural Resources Conservation Service as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans” (USDA NRCS, n.d.-a). The emphasis on the living, renewing nature of the soil microbiome is one reason why soil health has overtaken soil quality (which the NRCS defines as “the capacity of a soil to function for specific land uses or within ecosystem boundaries” (USDA NRCS, n.d.-b)) as a the leading conceptual framework to study soil ecosystems and inform sustainable nutrient management. Not all definitions of soil health use the same metrics: a recent paper (Devine et al., 2021) presents a conceptual framework for soil health assessment that divides soils into seven regions, each to be assessed differently, while another (Nunes et al., 2021) describes how SOC varies spatially across the US to inform interpretation of SOC values. Other soil health definitions may focus on specific goals, like the plant-pathology-centric version proposed by Janvier (2007), which emphasizes disease suppression characteristics.

Many of the appropriate soil health metrics for orchard agroecosystems are improved by OMAs by means of microbial mechanisms. Higher SOM and SOC are linked to many soil properties, especially soil structure (Lepsch et al., 2019; Mujdeci, 2011) and soil moisture (Lepsch et al., 2019; Villa et al., 2021; Walsh et al., 1996). OMAs have been shown to increase aggregate stability and decrease bulk density and compaction (Jahanzad et al., 2020; Lepsch et al., 2019; Mujdeci, 2011; Walsh et al., 1996). Aggregation relies on microbial compounds; as Six (2004) puts it, “when fresh plant material (as surface residues or roots) enters the soil, it induces the formation of aggregates because it stimulates the production of microbial-derived binding agents by being a C source for microbial activity” (p. 12). These changes improve soil structure. Surface-applied OMAs can also increase soil water content via the mulching effect, forming a physical layer which prevents moisture loss and buffers temperature (Jafari et al., 2012; J. Sanchez et al., 2003; Walsh et al., 1996), further stimulating the microbiome in a positive feedback loop (Lepsch et al., 2019).

OMAs have been shown to build up soil microbial biomass (Bonilla et al., 2012; Bossio et al., 1998; Jahanzad et al., 2020; López et al., 2014; Peck et al., 2011), increase the diversity of the soil microbiome (Bonilla et al., 2012, 2015; Volpiano et al., 2022), and sometimes induce disease-suppressive conditions through microbial community change (Bonilla et al., 2012, 2015; Janvier et al., 2007; Vida et al., 2016). Some OMAs can alter soil chemical properties such as pH (Andrews et al., 2021; López et al., 2014) and cation exchange capacity (Andrews et al., 2021; Khalsa et al., 2022; Villa et al., 2021), though not all OMAs have this effect on all soil types. OMAs are perhaps best known as a source of nutrients to replace or supplement other fertilizers. In addition to increasing soil carbon (Andrews et al., 2021; Khalsa et al., 2022; López et al., 2014; Peck et al., 2011; Villa et al., 2021), OMAs can provide large quantities of nitrogen, phosphorus, and potassium (Andrews et al., 2021; Khalsa et al., 2022; Villa et al., 2021), the first two of which are released via microbial substrate utilization. OMAs may increase the rate of nutrient solubilization from mineral sources because the added carbon may provide the energy needed to fuel these microbially-mediated reactions (Volpiano et al., 2022). Multiple studies have found OMA-derived nutrients alone to be a sufficient source of nutrients for a given orchard ecosystem (Baldi et al., 2010; Khalsa et al., 2022; López et al., 2014; J. Sanchez et al., 2003). Beyond supplying nutrients for the immediate growing season, repeated application of OMAs increases soil C and N storage (Clark et al., 1998; Khalsa et al., 2022; Mallory & Griffin, 2007), supporting the ability of the soil to meet nutrient demand in future years.

The Potential of Almond Hulls and Shells as an OMA

The nearly 2.5 million tons of almond hulls and shells (AHS) generated each year in CA (Fernandez-Bayo et al., 2020) have promise as a surface-applied organic matter amendment in orchards (Bonilla et al., 2015; Fernandez-Bayo et al., 2020; Jafari et al., 2012; López et al., 2014; Verdú & Mas, 2007; Vida et al., 2016). If AHS are to gain relevance as an OMA they must provide value in excess of that provided from secondary uses. Currently, in California almond hulls of the Non-Pareil variety can be sold as animal feed, fetching a price of $45-65/ton as of 2017 (Zuber et al., 2017), while hulls of other varieties and almond shells sell for little or nothing. The nutrients that can be released from AHS by the soil microbiome, however, may prove valuable, as may the many co-benefits to soil health, supporting the long-term sustainability of almond production systems in California's semi-arid landscapes.

A ten-year study in an organic avocado orchard showed that long-term repeated amendment with 7-cm layers of AHS created soil horizons more similar to those seen in forests than in conventional orchards (López et al., 2014). Other effects of AHS included increased microbiological activity and increased SOC, Kjeldahl N, and available P in the 0-25 cm soil horizon ranging from 48% to 110% above those of the control, with the OMA treatment providing sufficient nutrients to the trees and matching or exceeding yields in every year measured (López et al., 2014).

Complex effects of AHS OMAs on the microbiome and disease-suppressive qualities of the soil have also been observed (Bonilla et al., 2015; Vida et al., 2016). In another avocado system, AHS OMA increased the Shannon diversity index of the rhizosphere microbiome (Bonilla et al., 2015) and a disease-suppressive effect was observed that was attributed to changes in the soil microbiome, as indicated by the diminished or eliminated benefits when soil was heat-Pasteurized and restoration following inoculation of Pasteurized soil with fresh soil (Bonilla et al., 2015; Vida et al., 2016). While these assays focused on *R. necatrix*, a fungus that is pathogenic in avocados and wheat, researchers also identified diverse groups of organisms whose abundances increased after AHS amendment (Vida et al., 2016). Another study evaluated the effect of solarization and pathogen-inhibitory chemical compounds produced by AHS

OMAs, highlighting the generation of organic acids lethal to the pathogenic nematode *P. vulnus* (Fernandez-Bayo et al., 2020). Finally, two trials which tested AHS as a mulch (Jafari et al., 2012; Verdú & Mas, 2007) supported its use for water retention and weed suppression.

Nitrogen Dynamics Are Regulated by Microbial Biomass

Microbial response to OMAs is regulated by not only carbon but by nitrogen. Reliable sources of organic nitrogen are desireable since production of nitrogen fertilizer accounts for more than 50% of total energy use in commercial agriculture, according to one estimate (Woods et al., 2010), heavily contributing to global carbon emissions. Soil microbes are responsible for much of the nitrogen cycling that occurs in the soil and transformations into bioavailable pools for plant uptake. Soil microbial populations can also compete with plants for nitrogen uptake via immobilization into microbial biomass (Khalsa & Brown, 2019) with population turnover governing nitrogen release from this pool. Nitrogen immobilization is of concern as it may deprive orchard trees of N needed for growth (Khalsa & Brown, 2017); immobilization can however be valuable if it helps retain soil N during periods of low crop demand and thus can aid in preventing nitrate leaching (Chaves et al., 2007; Mallory & Griffin, 2007; J. Sanchez et al., 2003). Whether immobilization is desired in a particular agricultural site or not, the progression of nutrients through microbial biomass is crucial to the maintenance of the ecosystem (S. L. Jansson & Persson, 1982), since microbes drive decomposition and transform added nutrients into forms usable by the rest of the ecosystem while creating soil organic matter, increasing soil nutrient storage, and releasing microbially-derived compounds like those involved in aggregate formation.

The carbon-to-nitrogen (C:N) ratio of the OMA applied has a large impact on the fate of nitrogen added to the soil, with greater C:N values typically causing greater immobilization (Delin et al., 2012; Gale et al., 2006; Lazicki et al., 2020). This is because the bodies of microorganisms have their own C:N ratio, usually given as an average of 8:1 (Spohn, 2015; USDA NRCS, 2011), so in order to reproduce they must have adequate supply of both. Recent research, however, has complicated the idea that the C:N ratio of a substrate or the average C:N ratio of microbial biomass fully determine the fate of added nutrients. For example, when faced with a nitrogen-limited substrate microorganisms may alter their carbon use efficiency, allocating excess carbon to unexpected pools (Manzoni et al., 2008; Morrissey et al., 2017; Spohn, 2015). Since fungi usually have a higher C:N ratio than bacteria (Wallenstein et al., 2006), an overabundance of carbon relative to nitrogen may cause community shift instead of or in addition to immobilization of soil N by favoring higher C:N-containing organisms; as such, long term shifts in population structure could deeply impact the mineralization-immobilization dynamic with large implications for N retention and timing of nutrient release to plants. Predicting the outcomes of OMAs based solely on amendment C:N ratio is often inadequate and uncertain. A better understanding of the nutrient cycling dynamics of various OMAs across management gradients is necessary to inform predictive models.

Nutrient Mineralization: Modeling and Management

Finding ways to accurately predict the timing of nitrogen availability to plants would greatly improve the utility of OMAs to partially replace mineral fertilizers and reduce negative environmental impacts like nitrate leaching and fossil fuel consumption. This has been recognized as far back as 1926, when Löhnis wrote “In America, as economic conditions often favor green manuring, accurate knowledge of the results to be expected from the application of this method is highly desirable" (p. 253). Attempts at predictive models of nitrogen mineralization both with and without OMAs have been plentiful (Geisseler et al., 2019, 2021; Li et al., 2019; Liu et al., 2017; Osterholz et al., 2017) but realization has been elusive, and much work is still required before we can make reliable predictions. For example, Geissler (2021) called the performance of their own model "unsatisfactory" for two thirds of the OMA types described. Li (2019) highlights the complicated interactions between climatic factors like temperature and moisture and microbiological factors, showing that when microbial biomass was added to their model it became the primary driver and improved accuracy by 19%, but noting that microbial biomass is itself often influenced by climate, which determines the quality of the soil microbial habitat.

A further complicating factor when modeling mineralization from OMAs is the existence of the priming effect, in which adding an OMA to a system stimulates microbial activity and leads to the mineralization of additional carbon and nitrogen stored in the soil. This happens to both soil organic nitrogen (Fiorentino et al., 2019; S. L. Jansson & Persson, 1982) and soil organic carbon (Morrissey et al., 2017; Qiao et al., 2016). The result is that net N mineralization may be higher than the amount of N added by the amendment, suggesting that models may also need to consider soil C and N stocks. To provide the data needed for good predictive models, new methods for fast, affordable, and comprehensible measurements of the soil microbiome *in situ* throughout the immobilization-mineralization process are needed.

Research at UC Santa Cruz provides an example of optimized nitrogen management in which microbial immobilization is specifically designed to hold nitrogen in the organic fraction until crop N demand is higher (Hillman, 2020). In this system, strawberries are planted in the spring after a fall crop of brassicas has been harvested. Since the strawberries do not take up much nitrogen during their first few months of growth, nitrate in the soil is extremely susceptible to leaching with the winter rains, causing water pollution and financial losses. Researchers chose to apply a high C:N ratio amendment of finely ground almond shells to cause nitrogen immobilization until strawberry demand increased. The strategy delayed peak nitrogen availability, avoiding leaching, and increased strawberry yield (Hillman, 2020). This approach shows the potential to employ the soil microbiome to achieve precise nutrient storage and release while reducing negative environmental impacts. In this example, finding the right OMA and application rate was a matter of trial and error and was not driven by a modeling approach, and researchers acknowledge that it will take considerable time to develop predictable and replicable recommendations. Attempts at quantification of similar management techniques in a more generalized form have been without success (Chaves et al., 2007).

Impacts of Management Legacies on Microbiomes and OMA Turnover

Another interesting question related to OMA-based management of the soil microbiome is that of compounding effects, defined as occurring when the impact of treatments that happen in sequence is greater than, or simply different than, the sum of the two effects if the treatments were applied alone. Since every microbiome is unique in space and time, legacies from one management practice will impact the microbial community state and response to subsequent management. In practice, growers often implement multiple management practices sequentially at the same site, but this is rarely represented in controlled studies. There have been relatively few studies exploring how soil management history impacts response to subsequent inputs, and those which have looked at this question have not identified a clear trend. In general, the impact of larger stocks of soil organic C and N built up by years of organic management combined with the priming effect discussed above have greatly eclipsed any effect of microbial community composition or total microbial biomass. Most studies that looked at the fate of an added substrate to soils with divergent management histories have concluded that there was either no effect (Hadas et al., 1996; J. E. Sanchez et al., 2001; Stark et al., 2008) or a small effect of low relevance given the high rates of mineralization from soil organic carbon and nitrogen stores in the historically amended soils (Langmeier et al., 2002; Lazicki et al., 2020; Nett et al., 2012).

A 2007 study using soils with 13 years of divergent management history was able to differentiate between the effect of accumulated soil N stocks and the effect of the soil microbiome (Mallory & Griffin, 2007). It was observed that historically amended soil mineralized slightly less nitrate from an OMA source than the non-amended control, which the authors attributed to immobilization (Mallory & Griffin, 2007). However, this effect was much stronger when the OMA had a higher C:N ratio, a higher fibrous carbon content, and a stronger stimulating effect on microbial biomass. Researchers also observed faster mineralization in the historically amened soil during the first 7 days of incubation, attributable to higher soil microbial biomass, which agreed with previous findings (Franzluebbers et al., 1995). Lazicki (2020) agreed with Mallory and Griffin that management legacies on soil microbiomes can impact mineralization dynamics over time, highlighting the importance of considering accumulated nutrient pools when predicting immobilization-mineralization of OMA-derived N and the amount and timing of N availability.

Biostimulants vs. Microbiome Stimulants

Interest in the development of biostimulant products that enhance plant productivity by impacting the microbiome is growing rapidly (French et al., 2021). One criticism of these strategies is the observation that effects may be short-lived and disappear after a season or two, requiring growers to purchase biostimulants year after year to achieve desired effects. A review of studies on the effectiveness of microbial inoculants found that 86% of the inoculants modified soil microbial communities for some length of time (Mawarda et al., 2020) though the long-term benefits were mixed: of the subset that tracked long-term changes, 80% of studies reported shifts in microbial community composition but only a few found the inoculant organism alive at detectable levels (Mawarda et al., 2020). The authors provide four compelling mechanisms by which an inoculant organism might trigger community change (Mawarda et al., 2020), but community change driven by external environmental conditions is just as likely, especially when follow-up sampling was conducted in a different season than initial sampling. Multiple studies and reviews note that even strains of beneficial microorganisms that perform very well in lab or greenhouse trials are often outcompeted by native microbiota once scaled to field settings (Bacilio et al., 2017; French et al., 2021; Hungria et al., 2001; Sessitsch et al., 2019).

However, what are more likely to persist over time are changes to the soil microbiome that are driven by a shift in resource availability or local conditions (Fernandez et al., 2016; Volpiano et al., 2022) because these prompt the native microbiome to adapt to the new environment, with certain organisms enriched while others are outcompeted. It is these microbiome modifications, the ones that emerge because of environmental change, which may turn out to be the more promising intervention. OMAs influence the soil microbiome by changing both the physical habitat and the availability of resources. Understanding the mechanisms behind these effects is crucial because climate change threatens to subtly impact the microbiome in undesired directions (J. K. Jansson & Hofmockel, 2020). Paradoxically, climate change may make microbial interventions more viable: according to French (2021), "recent work suggests that first-generation microbiome manipulation may be more effective under stressed conditions (that is drought, heat and salinity)" (p. 260), which indicates an opportunity to harness the microbiome to ease the impacts of climate change on our crops, if we know how to do it.

Conclusion

Framing research questions around the soil microbiome, the driving force behind so many biogeochemical processes, could help close critical knowledge gaps and define new directions for research on organic matter amendments, nutrient mineralization management, and increasing agroecosystem resilience to disease and climatic stressors. Whether the goal is to improve the soil health of an orchard, to create a predictive model for precision management of plant nutrient application, or to come up with new strategies to meet old challenges, understanding microorganism community assembly and dynamics in response to management is key. Cutting-edge research on these issues brings us closer to the imagined future of engineering microbiomes to meet human needs safely and sustainably.

Chapter 2: Evaluation of Almond Hull and Shell Amendment Across Soil Management Gradients

Introduction

Research conducted in 2019-2021 explored the use of almond hulls and shells (AHS) as an organic matter amendment (OMA) in almond orchards. With nearly 2.5 million tons of almond hulls and shells generated each year (Fernandez-Bayo et al., 2020) from the 1.6 million acres of almonds in California (USDA, 2021), finding a productive use for these materials would be beneficial. Previous trials on AHS have taken place in avocado, fig, and citrus orchards (Bonilla et al., 2015; Jafari et al., 2012; López et al., 2014; Verdú & Mas, 2007), but trials in almond orchards are needed. Muhammad et al. (2015) found that the percentage of whole tree annual nutrient demand that went to almond hulls, shells, and kernels combined was 90% of N, 87% of P, 91% of K, 86% of S, 90% of Ca and 87% of Mg. Recapturing these nutrients could considerably reduce growers' fertilizer budgets and potentially mitigate the environmental impacts of mineral fertilizers.

There is a growing interest in off-ground harvest, also known as catch-frame harvest or advanced harvest, as one of a set of new practices improving the sustainability of almond orchards. Conventional on-ground harvest requires orchard alleys to be bare and dry at the time of harvest and limits options for improving soil health. Repeated machinery passes cause compaction and surface disturbance, damaging soil structure and generating dust, a serious air quality hazard that negatively affects nearby residents and farmworkers (Gill, S., 2018; Schenker et al., 2009) and results in topsoil loss. Catch-frame harvesters utilize fewer machinery passes and do not require bare alleys, enabling a wider range of soil health practices. The more efficient cycling of nutrients, improvements in soil health, and reduction of dust as a health hazard that would occur with the adoption of off-ground harvest could vastly improve the sustainability of the almond industry in California and elsewhere.

We paired a field trial in a commercial almond orchard with a 210-day soil incubation to explore how C and N mineralization and the soil microbiome were affected by combinations of three soil health practices: green waste compost amendment, almond hull and shell amendment, and catch-frame harvest. While our field trial centered on characterizing impacts of almond hull and shell amendment and catch-frame harvest in a conventional almond orchard, our incubation trial examined the long-term impacts of green waste compost amendment on almond hull and shell decomposition and C and N release as well as microbial respiration and microbial biomass. Incubations have been used to look at a variety of biogeochemical processes when a setting without plant uptake or routine fertilizer application is desirable, including carbon and nitrogen mineralization, CO_2 evolution, and microbial enzyme production (Fernandez-Bayo et al., 2020; Franzluebbers et al., 1995; Geisseler & Horwath, 2009; Hart et al., 1994; Khalsa et al., 2016; Lazicki et al., 2020; Xiang et al., 2008).

In the field trial conducted here, we hypothesized that both AHS amendment and catch-frame harvest (which can be considered a soil treatment in that it reduces disturbance) would increase soil health indicators such as soil organic carbon, soil nitrogen, and soil microbial biomass. In the incubation trial, we hypothesized that AHS amendment would increase soil health indicators such as soil organic carbon, soil nitrogen, and soil microbial biomass but that there would be subtle differences in how the two soils with differing management histories responded. We hypothesized that the already larger C and N pools of the green waste compost treated soil might increase more compared to the no compost soil in response to the addition of AHS and that the same might occur with total microbial biomass, potentially causing some immobilization of soil nitrogen.

Materials and Methods

Experimental Conditions

The effect of soil management history on C and N mineralization under a subsequent amendment was examined in soil that had received a green waste compost amendment for three years (2015-2017) as part of a previous field trial (Khalsa et al., 2022; Lepsch et al., 2019). This soil was collected from an almond orchard located near Escalon, CA, in San Joaquin County, USA (37°49’33"N 121°6’45"W) where the soil was a Manteca fine sandy loam (coarse-loamy, mixed, superactive, thermic Haplic Durixeroll). Samples were taken in fall of 2020 from a depth of 0-10 cm from 8 rows. Four rows had received surface-applied green waste compost (GWC) while four had served as a control and samples were taken from flagged locations used in the previous study, one per row (Khalsa et al., 2022). Soils were aggregated by treatment, air-dried, sieved to 4 mm, and weighed into 96 1-quart glass mason jars in aliquots of 615 g, reaching a depth of 7.6 cm. The 96 jars were arranged in a completely randomized design, with 48 receiving each of the two soils. Almond hulls and shells were obtained from Mariani Nut Company in Winters, CA, oven-dried, and partially ground by hand using a mortar and pestle. 24.3 g of AHS amendment was added to 24 jars of each soil, separated from the soil surface by a thin, flexible mesh layer (Figure 1). There were therefore 24 replicates of each combination of soil management history and AHS amendment. On each of the planned sampling dates, 4 replicates of each treatment combination were removed. Levels of amendment were almond hull and shell amended (AHS) or unamended (UA) and levels of history were green waste compost (GWC) or no compost (NC) (Table 1).

The incubation trial ran for 210 days. On Day 1 of the incubation, 120 mL of de-ionized (DI) water was added to each jar to re-wet the soil up to field capacity, which had been determined to be ~19% volumetric water content in a previous study (Lepsch et al., 2019). Water was added a few mL at a time to avoid creating preferential flow paths. An additional 15 mL of DI water was added to amended jars to re-wet the amendment layer to its capacity, which was determined by wetting samples of oven-dry amendment, letting them drain, and recording the weight of water held. The amount of water added for the amendment layer was adjusted throughout the incubation when new dry masses were recorded, while the amount of water added for the soil was kept constant. DI water was added weekly by weight to bring the jars to field capacity. Jars were kept in a temperature- and humidity-controlled room located in the UC Davis Post-Harvest Lab, without lids (aerobically), for the duration of the incubation. The temperature was 20 °C and the relative humidity was 60% (+-2%). The room was dark except when someone was working in it.

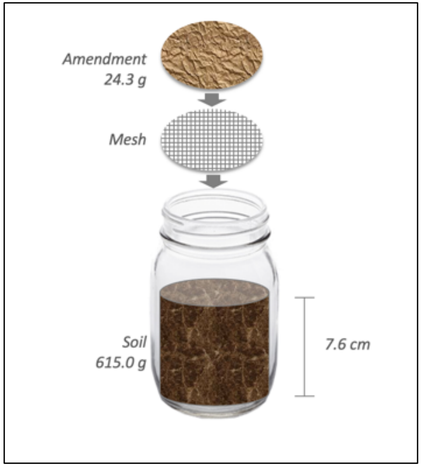


Figure 1. Diagram showing how soil and AHS amendment were placed in jars in the incubation trial.

The field trial was established in 2019 at a mature almond orchard located in Woodland, CA, in Yolo County, USA (38°40'12"N -121°53'40"W) on a San Ysidro clay loam (fine, smectitic, thermic Typic Palexeroll) using a split-plot randomized complete block design with 4 blocks and AHS amendment forming the main plots. Almond hull and shell amendment from Mariani Nut

Company in Winters, CA was applied to treatment plots at a rate of approximately 18 tonnes/ha (8 tons/acre) in fall of 2020. The amount of AHS used in the incubation trial was calculated to match this application rate, and the AHS used in both trials were from the same shipment and source. The C:N ratio of the amendment was approximately 52:1, consisting of on average 44.5% carbon and 0.8% nitrogen. Harvest took place in late summer of 2021 and both catch-frame and on-ground harvest machinery were used in their respective plots. Irrigation and fertilization followed the grower's standard regimen and were recorded (not reported in this paper). In addition to being described here, this field trial was part of a larger project and additional measurements will be reported in future work. Levels of amendment were almond hull and shell amended (AHS) or unamended (UA) and levels of harvest were catch-frame harvest (CFH) or traditional harvest (TH) (Table 1).

Treatment Abbreviations	
AHS	Almond hull and shell amended
UA	Unamended
GWC	Green waste compost history
NC	No compost history
CFH	Catch-frame harvest
TH	Traditional harvest

Table 1. Treatment abbreviations.

Sample Analysis

Sampling Procedure

For the incubation trial, baseline measurements were performed on 8 field samples taken from a depth of 0-10 cm just prior to the start of the incubation, one from each row of the previous trial. All other measurements were performed on 16 samples in accordance with the design of the incubation trial. At Days 15, 30, 45, 60, 120, and 210, 16 jars, 4 replicates of each of the 4 treatment combinations, were destructively sampled and subsamples of soil were allocated to each assay. Watering was timed to occur 4 days before sampling dates. Amendment residue was removed from the soil surface at the beginning of sampling by lifting the mesh out of the jar and scraping any material above the mesh into a vessel to be analyzed separately. Soil was emptied onto a metal tray and subsamples were weighed into the appropriate vessels, with care taken to include soil from 4-5 random places on the tray in each subsample. Remaining soil was brushed away and the process was repeated for each of the 16 jars. Sets of subsamples were: air-dried (bulk C and N, ~200 g), oven-dried (moisture content, 30-40 g), refrigerated before further processing (microbial biomass, dissolved organic carbon, and mineral N, 12 g), or frozen at -80 C (sequencing, results not reported in this paper).

For the field trial, soil sampling was primarily done in September 2020, April 2021, and October 2021, with additional measurements, such as amendment residue collection from litter bags, performed at more frequent intervals. Soil samples were taken from a depth of 0-10 cm using a Dutch auger at three points in each treatment row and aggregated by row, then kept on ice until returned to the lab where they were sieved to 4 mm and subsamples were allocated to each assay as above. There were 4 replicates of each of the 4 treatment combinations, one from each block.

Cumulative CO_2

CO_2 samples were taken from the incubation trial at Days 15, 30, 60, 90, 120, and 210. Jars were first tightly sealed using metal lids with septa. At 0, 30, and 60 minutes, a syringe was used to mix headspace gas and transfer 20 mL of it into evacuated exetainers. Gas samples were analyzed using a LI-COR LI-6251 CO_2 Analyzer. 1 mL of each gas sample was injected into the machine using a syringe and the peak reading was recorded. A set of three standards were run every 16 samples. Raw readings were transformed to parts per million using standard curves, which were unique for each run and were fit to all standard values from that run. Values were converted to a per gram of dry soil basis based on 615 g dry soil per jar. Total microbial respiration was calculated as a rate of CO_2 evolution over the hour measured and extrapolated between measurement dates. These rates were used to calculate cumulative CO_2 evolution over time piecewise using trapezoidal areas. CO_2 emissions due to the amendment were calculated as the difference between cumulative CO_2 evolution from amended and unamended treatments.

Bulk Soil and Amendment Layer

Subsamples of soil from the baseline and final sampling dates were air-dried, ground, and sent to the UC Davis Analytical Lab for quantitative determination of total organic carbon (*Total Organic Carbon - Combustion Method*, 2017) and total nitrogen (*Total Nitrogen And Carbon - Combustion Method*, 2017). Amendment residue recovered from the incubation as described above was oven-dried and aggregated by sampling date. Field amendment residue was collected from litter bags in the AHS-CFH treatment rows at 30, 60, 120, 150, 240, 293, 365 days of decomposition and oven-dried. Amendment decomposition rates were determined by mass of oven-dry samples before aggregation in the incubation trial and similarly in the field trial. For Day 15 of the incubation trial only, an error was made in which samples were dried in pairs within the same treatment and averages were taken. Subsamples from both sets of amendment layer residue were sent to the UC Davis Analytical Lab for quantitative determination of total carbon and nitrogen (*Total Nitrogen and Carbon-Combustion Method*, 2017). Because incubation trial amendment residue was aggregated by sampling date after weighing, independent replicates for amendment total C and N were lost and standard error is reported instead.

Mineral Nitrogen, Dissolved Organic Carbon, and Microbial Biomass

For both the incubation trial and the field trial, to obtain ammonium and nitrate N, dissolved organic C (DOC), and microbial biomass C (MBC) and N (MBN), two subsamples of 6 g of fresh soil were refrigerated from sampling until processing, no more than one week. One subsample was fumigated with chloroform for 24 hours while the other remained unfumigated (Horwath & Paul, 1994). Both fumigated and unfumigated subsamples were then extracted by adding 30 mL 0.5 M K_2SO_4, shaking on a shaker for one hour, and filtering through Fisher brand Q5 filter paper (Horwath & Paul, 1994; Mulvaney, 1996). Soil extracts were frozen until use. For MBC, MBN, and DOC, 8 mL of each soil extract was diluted with 32 mL of de-ionized water and sent to UC Merced for analysis with a Shimadzu TOC-Vcsh TOC Analyzer to obtain non-purgeable organic carbon and total extractable nitrogen. Standards were included with runs and readings were transformed using standard curves. A check standard was run every 12 samples.

Resulting values were blank-adjusted. Microbial biomass was calculated as the difference between fumigated and unfumigated samples. A conversion factor of 1/0.45 was used for MBC and 1/0.54 for MBN (Brookes et al., 1985). Values were converted to a per gram of dry soil basis by multiplying by a volume to soil ratio of 25 and by 1 + the sample gravimetric moisture content. Data showed some possible contamination of samples or blanks, causing us to drop some time points from the data set. Some values appeared to be negative, which may have been the result of statistically expected experimental error since these pools, especially MBN, are very small, but contamination or other sources of error cannot be ruled out. A few (<5) samples were missing due to spillage and were half of a fumigated-unfumigated pair. These were addressed by calculating the average fumigated:unfumigated ratio for that time point and using it to replace the missing value to obtain a microbial biomass difference. These back-calculated values were not used in the DOC data. DOC values were considered equivalent to non-purgeable organic carbon in the unfumigated samples and were also converted to a per gram of dry soil basis by multiplying by a volume to soil ratio of 25 and by 1 + the sample gravimetric moisture content.

For the incubation trial only, remaining soil extract was analyzed for ammonium and nitrate concentration using colorimetric assays (Doane & Horwath, 2003; Verdouw et al., 1978). Reagents and standards were mixed by hand and solutions were added to microcuvettes in duplicate using micropipettes. Microcuvettes were run on a Thermo Scientific Genesys 10S UV-Vis Spectrophotometer. Standards and blanks were included with each run of samples, also in duplicate. Readings were transformed using standard curves that were unique for each run. Technical replicates were averaged to obtain one value per sample. Values were blank-adjusted. Values were converted to a per gram of dry soil basis by multiplying by a volume to soil ratio of 5 and by 1 + the sample gravimetric moisture content. Since ammonium concentrations were low, we will report the combined concentration of ammonium and nitrate as mineral N. Regression lines for the rates at which observed mineral N increased were fitted for each replicate. Fits with an R^2 value of greater than 0.85 were accepted.

Statistical Analysis

Data pre-processing was performed in Microsoft Excel. Statistical analysis was performed using R 4.1.1 (R Foundation for Statistical Computing) to assess the effect of History, Amendment, and Time for the incubation trial or Harvest, Amendment, and Time for the field trial on each response variable. Analysis of variance (ANOVA) was used with fixed effects (History, Amendment, and Time or Harvest, Amendment, and Time) and all interaction terms. Time was treated as a categorical variable because measurements were taken from different jars at each time point and therefore there was no correlated variance structure. Linear models were created and a three-way ANOVA on each model was used to test for significant differences of fixed effects. Residuals were tested for homogeneity and normality using Quantile-Quantile Plots and Scale-Location Plots. Multiple pairwise comparisons of least square means were performed using the emmeans package, which applied the Tukey method of p-value adjustment with a significance level of alpha=0.05. For amendment layer data only, p-values were adjusted using the Dunnett method or were not adjusted, as appropriate.

Results

Bulk Soil C and N

Starting values of total organic carbon (TOC) and total nitrogen (TN) at the beginning of the incubation were nearly twice as high for the GWC soil as for the NC soil (Table 2). Estimated means of C and N content for these baseline GWC and NC soils were significantly different (p<0.001), 1.21% and 0.12% for the GWC soil versus 0.59% and 0.06% for the NC soil, respectively. Soil TOC and TN remained roughly the same at the end of the incubation with slight increases that were not significant. We are still awaiting

	GWC	NC
Baseline TOC	1.21 %	0.59 %
Final, Amended TOC	1.31 %	0.52 %
Final, Unamended TOC	1.33 %	0.53 %
	GWC	NC
Baseline TN	0.12 %	0.06 %
Final, Amended TN	0.14 %	0.06 %
Final, Unamended TN	0.14 %	0.06 %

Table 2. Soil TOC and TN in the incubation trial. Differences between soil with a history of green waste compost (GWC) versus no compost (NC) were significant throughout, while all other differences were not.

bulk soil TOC and TN from our field trial. Samples from 0, 200, and 365 days have been submitted.

Cumulative CO_2

Cumulative CO_2 evolution from microbial respiration was significantly affected by both AHS amendment and soil management history, but the impact of the AHS amendment was larger. AHS amendment increased final cumulative CO_2 evolution by an estimated 5.618 mg CO_2 per g dry soil on the GWC soil ($p<0.0001$) and 4.194 mg CO_2 per g dry soil on the NC soil ($p<0.0001$) (Table 3). Having a history of GWC, meanwhile, increased final cumulative CO_2 evolution by an estimated 2.233 mg CO_2 per g dry soil when amendment was present ($p<0.0001$) and 0.809 mg CO_2 per g dry soil when no amendment was present ($p<0.001$). There was also an interaction effect between soil history and AHS amendment ($p<0.001$). The AHS amendment more than tripled cumulative CO_2 evolution on the GWC soil but more than quadrupled it, although lower overall, on the NC soil.

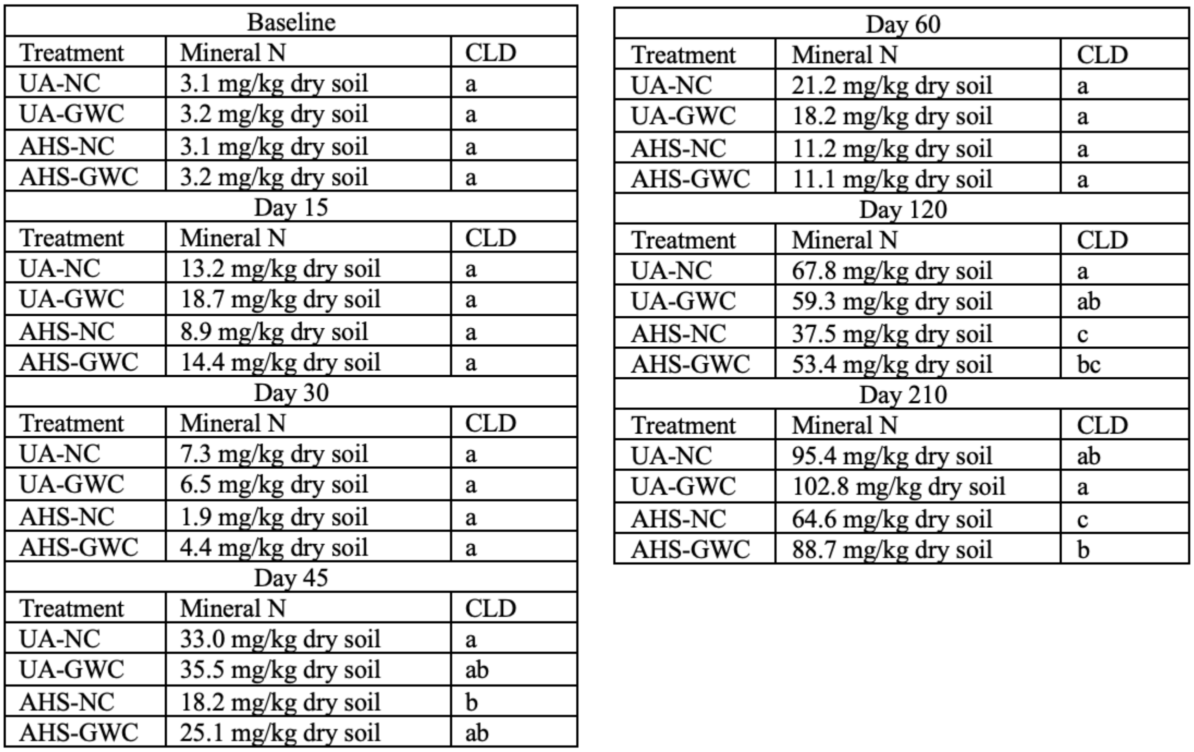
Treatment Contrast	Estimated Difference	p-value
AHS Amendment & GWC History – AHS Amendment & No Compost History	2.233 mg CO_2/g dry soil	<0.0001
AHS Amendment & GWC History – No AHS Amendment & GWC History	5.618 mg CO_2/g dry soil	<0.0001
AHS Amendment & GWC History – No AHS Amendment & No Compost History	6.427 mg CO_2/g dry soil	<0.0001
AHS Amendment & No Compost History – No AHS Amendment & GWC History	3.385 mg CO_2/g dry soil	<0.0001
AHS Amendment & No Compost History – No AHS Amendment & No Compost History	4.194 mg CO_2/g dry soil	<0.0001
No AHS Amendment & GWC History – No AHS Amendment & No Compost History	0.809 mg CO_2/g dry soil	<0.001

Table 3. Estimated differences in final (Day 210) cumulative CO_2 evolution by treatment in the incubation trial. AHS is almond hulls and shells and GWC is green waste compost.

Mineral N

Mineral nitrogen accumulation was substantial across treatments: at their peak at Day 210, estimated mean values of mineral N ranged from 65-103 mg per kg dry soil (Table 4). The amount of mineral N observed in the soil was significantly affected by both AHS amendment ($p<0.0001$) and soil management history ($p<0.0005$). All treatments showed net N mineralization and increasing amounts of mineral N with time, but the quantities in the unamended treatments were slightly higher. Differences among treatments were significant at Days 45, 120, and 210. The AHS-NC treatment, in particular, displayed less net N mineralization than other treatments.

The interaction effect between AHS amendment and soil history ($p<0.005$) is of particular interest. Peak mineral nitrogen, at Day 210, was lowest in the AHS-NC treatment, significantly lower than the three other treatments ($p<0.0001$ for all three comparisons), while the N release in the AHS-GWC treatment was significantly lower than in the UA-GWC treatment ($p<0.005$) but similar to in the UA-NC treatment ($p=0.3632$) (Table 4). When the rates at which observed mineral N increased were calculated, the rate for the AHS-NC treatment was significantly lower ($p<0.005$) than each of the other three, which were not distinguishable (Figure 2). This result demonstrates that, over the period of measurement, a soil receiving an OMA for the first time may see reduced net N mineralization compared to a soil with a history of OMAs.



Baseline		
Treatment	Mineral N	CLD
UA-NC	3.1 mg/kg dry soil	a
UA-GWC	3.2 mg/kg dry soil	a
AHS-NC	3.1 mg/kg dry soil	a
AHS-GWC	3.2 mg/kg dry soil	a
Day 15		
Treatment	Mineral N	CLD
UA-NC	13.2 mg/kg dry soil	a
UA-GWC	18.7 mg/kg dry soil	a
AHS-NC	8.9 mg/kg dry soil	a
AHS-GWC	14.4 mg/kg dry soil	a
Day 30		
Treatment	Mineral N	CLD
UA-NC	7.3 mg/kg dry soil	a
UA-GWC	6.5 mg/kg dry soil	a
AHS-NC	1.9 mg/kg dry soil	a
AHS-GWC	4.4 mg/kg dry soil	a
Day 45		
Treatment	Mineral N	CLD
UA-NC	33.0 mg/kg dry soil	a
UA-GWC	35.5 mg/kg dry soil	ab
AHS-NC	18.2 mg/kg dry soil	b
AHS-GWC	25.1 mg/kg dry soil	ab

Day 60		
Treatment	Mineral N	CLD
UA-NC	21.2 mg/kg dry soil	a
UA-GWC	18.2 mg/kg dry soil	a
AHS-NC	11.2 mg/kg dry soil	a
AHS-GWC	11.1 mg/kg dry soil	a
Day 120		
Treatment	Mineral N	CLD
UA-NC	67.8 mg/kg dry soil	a
UA-GWC	59.3 mg/kg dry soil	ab
AHS-NC	37.5 mg/kg dry soil	c
AHS-GWC	53.4 mg/kg dry soil	bc
Day 210		
Treatment	Mineral N	CLD
UA-NC	95.4 mg/kg dry soil	ab
UA-GWC	102.8 mg/kg dry soil	a
AHS-NC	64.6 mg/kg dry soil	c
AHS-GWC	88.7 mg/kg dry soil	b

Table 4. Estimated mean mineral N concentrations in the incubation trial. Treatment abbreviations are almond hull and shell amended (AHS) or unamended (UA) and green waste compost (GWC) or no compost (NC). Compact letter displays show groups that are significantly different from one another at a threshold of $p < 0.01$.

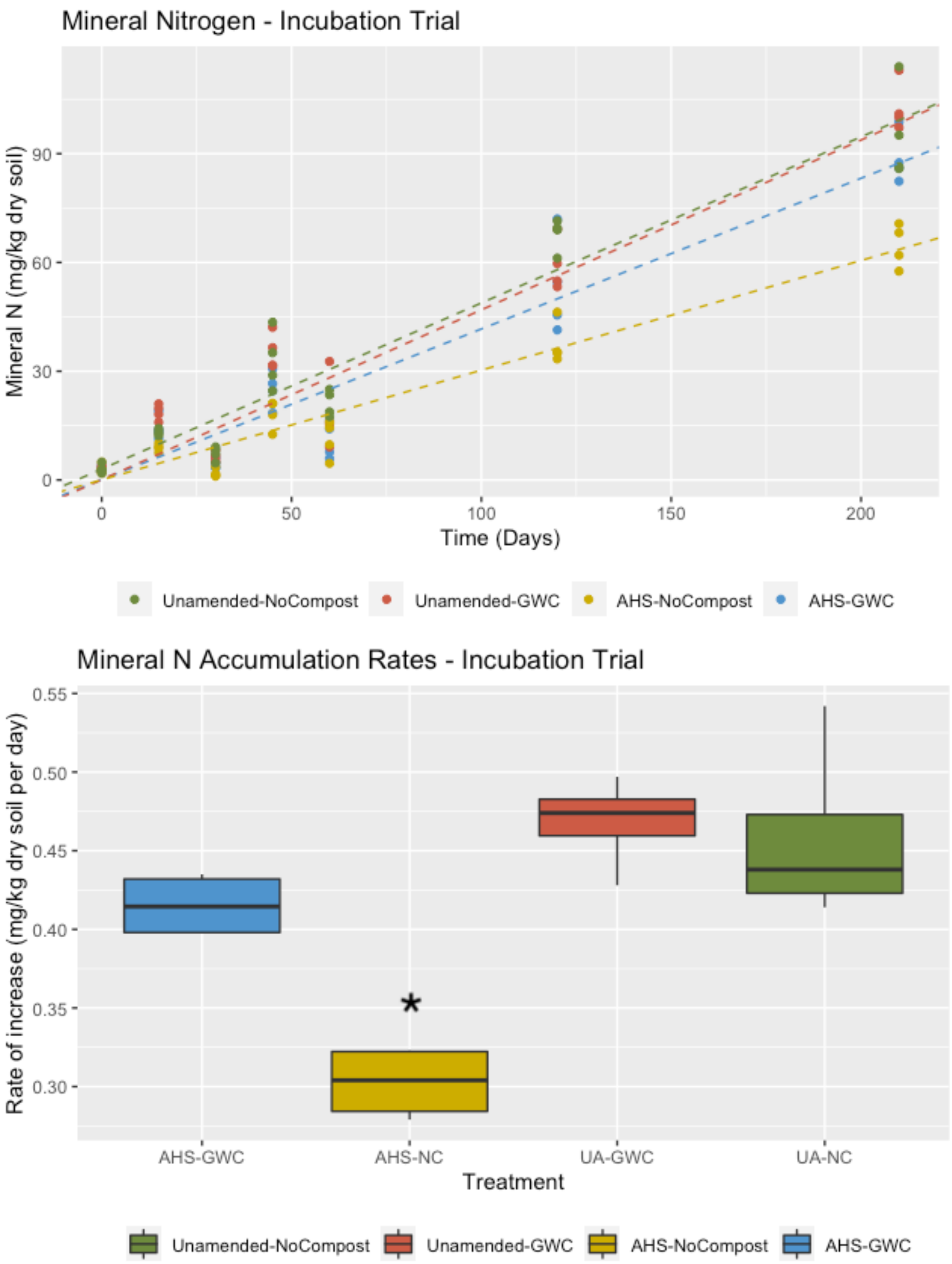


Figure 2. Top: Observed values of mineral nitrogen with lines representing the average rates of increase for each treatment overlaid. Bottom: Rates of increase in observed mineral nitrogen by treatment (n=16). The * indicates this treatment is significantly different from the other three ($p<0.005$). AHS is almond hulls and shells and GWC is green waste compost.

Dissolved Organic Carbon

In the incubation trial, soil management history had a strong effect on dissolved organic carbon (DOC) ($p<0.0001$). Baseline DOC was not significantly different among treatments, but by Day 30, DOC in the AHS-GWC and UA-GWC treatments became significantly higher than in the AHS-NC and UA-NC treatments ($p<0.05$) and was on an upward trend (Figure 3). By Day 210, DOC values in the GWC treatments were roughly double those of their NC counterparts ($p<0.005$ for the differences). Averaged over time, a history of GWC increased DOC by an estimated 29.1 micrograms per g dry soil when AHS amendment was present and 29.8 when it was not ($p<0.0001$ for both).

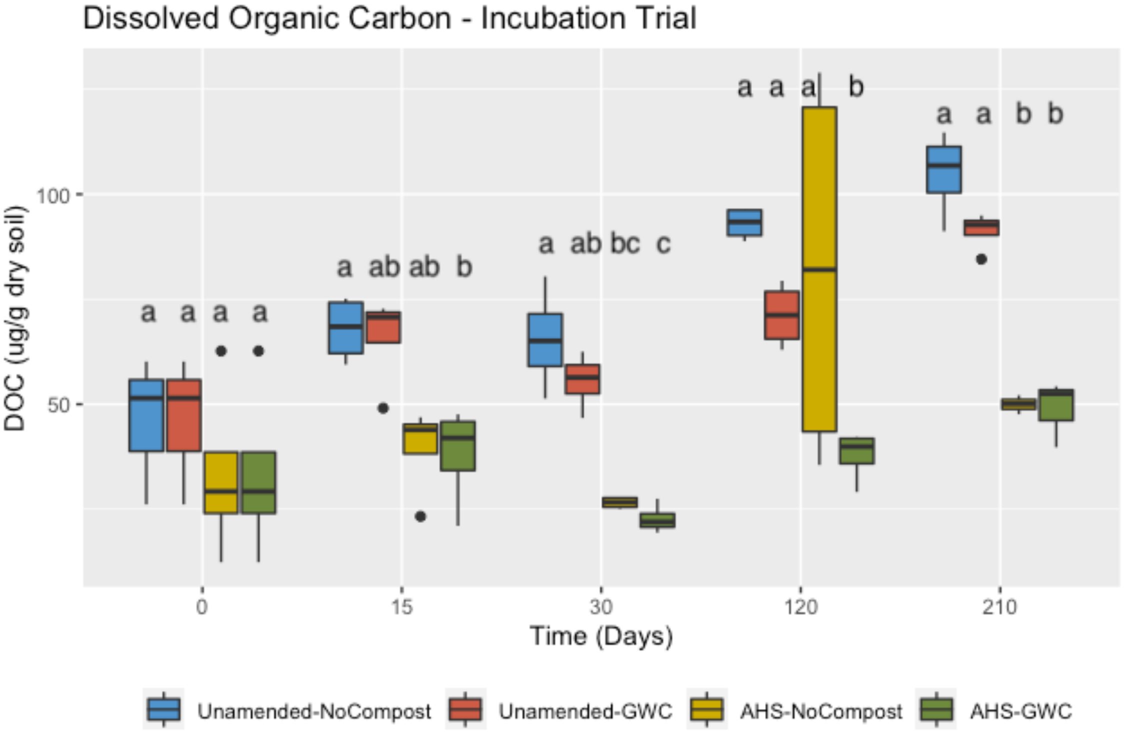


Figure 3. Dissolved organic carbon in the incubation trial. Letters show groups that are significantly different from one another at a threshold of $p < 0.05$. AHS is almond hulls and shells and GWC is green waste compost.

The effect of the AHS amendment was also significant in the ANOVA ($p<0.01$) but was smaller than the effect of history and when averaged over time the increases in DOC from amendment alone were no longer significant. Both soil history and AHS amendment interacted with time, but while the impact of soil history increased over time, the impact of AHS amendment was significant only at Day 120. There was no significant amendment-history interaction here as there was elsewhere.

In the field trial, DOC was low throughout the year, increasing slightly in the spring and dropping again by the following fall. This is not surprising a California climate, especially during a drought. Time produced the only significant effect.

Amendment Layer

After 210 days, the incubation trial amendment had lost 37.8% of its dry mass on average. Decomposition was fastest in the first 30 days and slowed to a steady pace thereafter. This aligns surprisingly well with what we saw in the field, where the amendment had lost 42.4% of its dry mass after 240 days and 55.2% after one year. A linear regression on average dry masses (Figure 4) suggests that the pace of decomposition was 0.17% per day in the laboratory incubation ($R^2=0.839$) and 0.13% per day in the field trial ($R^2=0.907$). These similar decomposition rates despite the differing soil taxonomies and temperature and moisture regimens (our field trial experienced the full range of seasons while our incubation modeled winter conditions for its entirety) mean that the data may describe a characteristic intrinsic to the amendment and may be useful for predicting decomposition rates across sites.

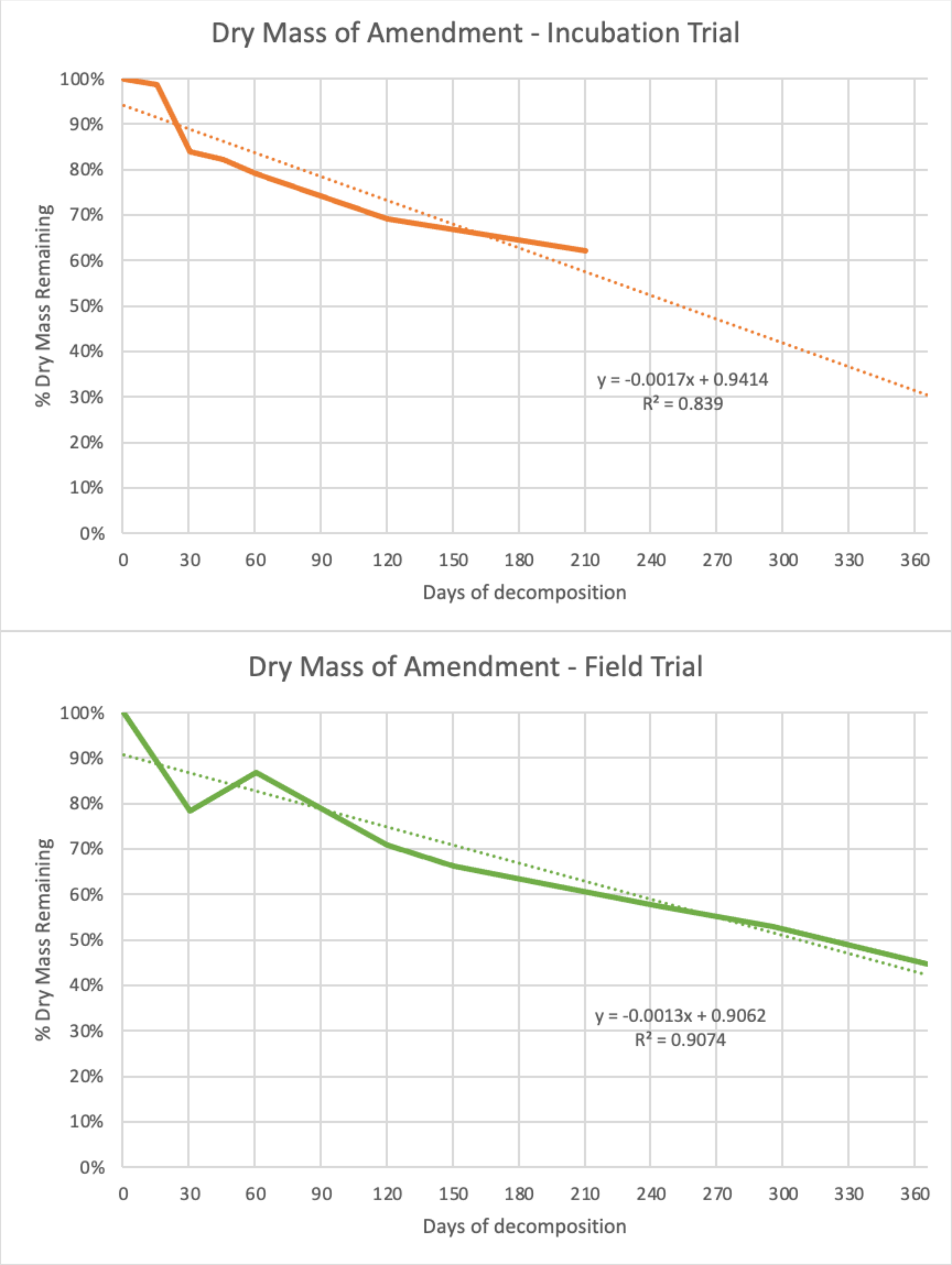


Figure 4. Amendment decomposition rates in the two trials. Solid lines connect observed data points. Dotted lines represent linear regressions with the equations and R^2 values given.

Soil history appeared to cause significantly more amendment layer decomposition on average over the course of the incubation, but the effect size was very small, an estimated 0.44 g more lost on GWC soil compared to NC soil ($p<0.05$), or an extra 1.8% of the initial mass. Differences in dry mass remaining were only significant at Days 45 and 210 (not Days 15, 30, 60, or 120) and the rates of decomposition broken out by soil history, as represented by the slopes of linear regression lines, were not significantly different, leading us to discount this effect.

In the incubation trial, total carbon (TC) concentration in the amendment layer was unchanged throughout most of the experiment, showing no major differences. TC in the field trial seemed to increase at 30 days and decline thereafter, with the values at 0 days and 365 days not significantly different from one another. By contrast, incubation total nitrogen (TN) concentration increased to 1.19% by Day 210, 0.25 percentage points above the initial measurement (SE=0.039) and 0.19 above Day 120 (SE=0.032). We observed a similar trend in the field trail, where TN increased steadily over time with the difference from the baseline first becoming significant at 150 days ($p<0.005$) (Table 5). By 365 days, TN in the field trial had increased by about three-quarters of a percentage point ($p<0.0001$). It seems likely that amendment TN in both trials followed the same pattern, but the timing of sampling was such that in the incubation trial only one elevated value was observed.

Days of Decomposition	Amendment TN Incubation Trial	Standard Error	Amendment TN Field Trial	p-value (difference from time 0)
0	NA	NA	0.85 %	
15	0.94 %	0.0392	NA	NA
30	1.00 %	0.0554	0.90 %	0.9560
45	0.97 %	0.0554	NA	NA
60	1.01 %	0.0554	0.91 %	0.9305
120	1.00 %	0.0554	0.96 %	0.6072
150	NA	NA	1.17 % †	<0.005
210	1.19 %	0.0320	NA	NA
240	NA	NA	1.34 % †	<0.0001
293	NA	NA	1.50 % †	<0.0001
365	NA	NA	1.60 % †	<0.0001

Table 5. Total nitrogen content of amendment residue over time. †Significantly different from time 0 in the field trial.

Microbial Biomass

One unanticipated effect in our incubation trial was the steep decline of microbial biomass over time. In retrospect, the lack of plants and the mostly dark conditions in the room meant that there was little if any addition of photosynthates to the system, likely resulting in the collapse of some microbial populations in the latter part of the experiment. Nonetheless, broad effects can be seen (Figure 5). For MBC, the ANOVA reported effects of both soil history ($p<0.01$) and AHS amendment ($p<0.1$). Soil history had the stronger effect because the estimated baseline value for the GWC soil was more than double that of the NC soil ($p<0.1$ for the difference). For MBN, the ANOVA reported more confidence in the effect of AHS amendment ($p<0.005$) than soil history ($p<0.1$). Baseline MBN for the GWC soil was also more than double that of the NC soil but the difference was not significant ($p=0.534$). Although the ANOVAs detected differences, few of the direct comparisons at individual time points returned significant p-values.

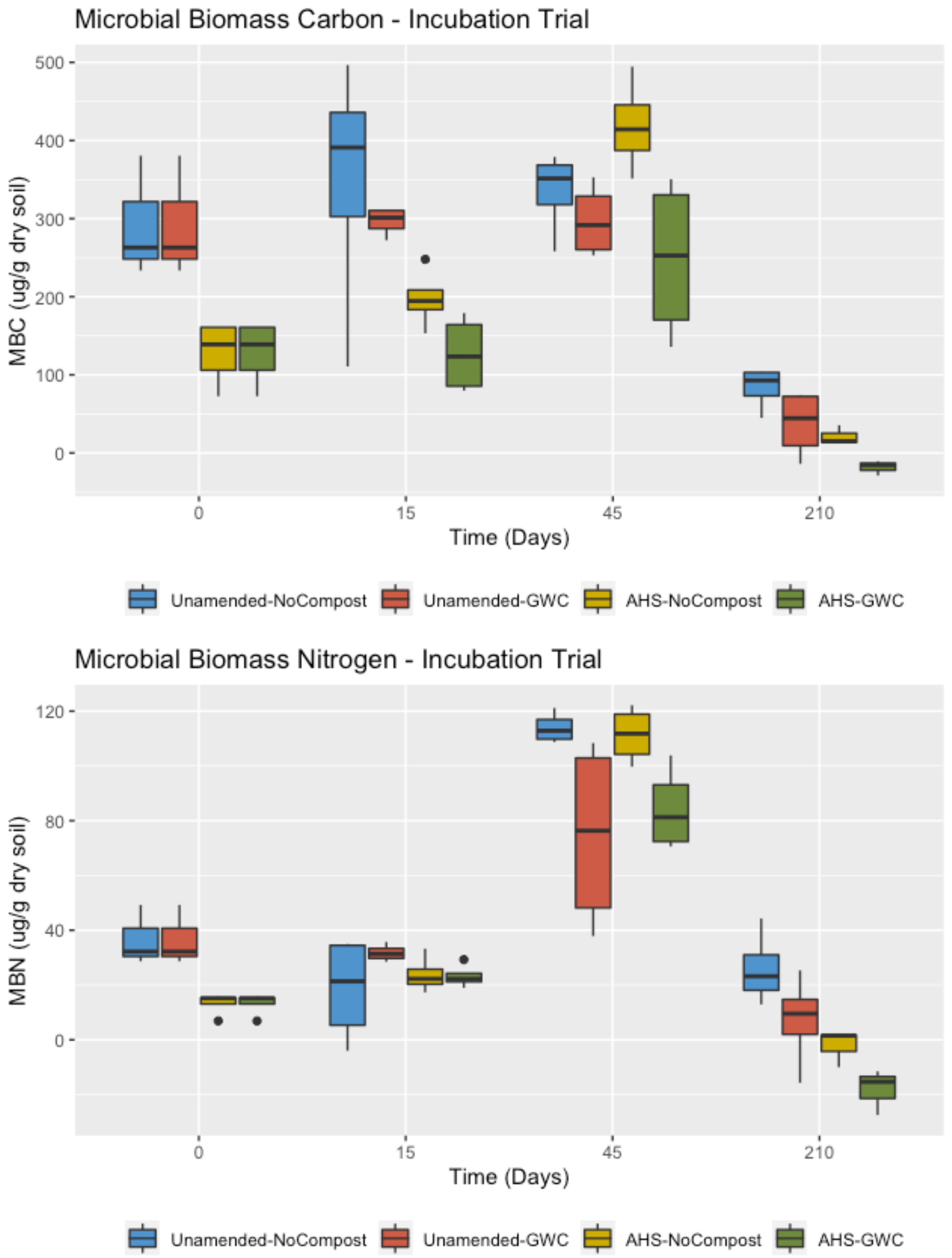


Figure 5. Microbial biomass C and N in the incubation trial. Most direct comparisons at specific timepoints were not significant. The effect of soil history can be seen in the baseline values, however, and the AHS amendment seemed to lead to increases at Day 45. AHS is almond hulls and shells and GWC is green waste compost.

In our field trial, we were able to get a clearer picture of the microbial biomass trends. Estimated baseline values of MBC across all treatment plots, taken in fall, were around 175-215 micrograms per g dry soil with no significant differences (Figure 6). In spring, MBC was higher in all treatments and was greatest in the two UA treatments compared to the two AHS treatments ($p<0.005$). This effect on MBC appeared transient, however, because by the one-year point that fall the trend had reversed, and the two AHS treatments had higher MBC than the two UA treatments ($p<0.0001$) by a wider margin (an estimated difference of 248.2 micrograms per g dry soil in fall compared to a difference of 160.8 in spring). Changes in MBN were for the most part not significant. When run for all three time points, an ANOVA found no treatment effect on MBC other than time, but when run for the one-year point alone, an ANOVA found a significant effect of amendment ($p<0.0001$) and an effect of harvest strategy that was not significant ($p=0.1426$).

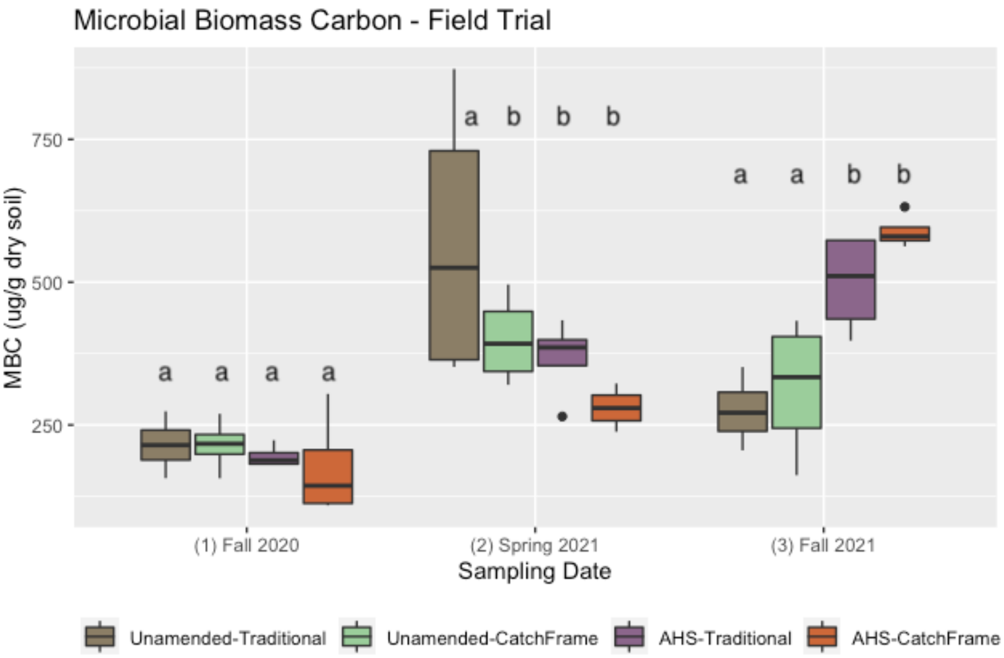


Figure 6. Microbial biomass carbon in the incubation trial. Letters show groups that are significantly different from one another at a threshold of $p < 0.1$. AHS is almond hulls and shells.

Discussion

Bulk Soil C and N

The impact of soil management history was apparent before the incubation began, as soil C and N stores reflected past management decisions. The fact that three years of compost application still had such an effect three years after input had stopped highlights the lasting impact of OMAs as well as the importance of taking management history into account when assessing an agricultural ecosystem.

While we did not see significant changes to these pools from the AHS amendment over the 210 days of the incubation, López (2014) previously demonstrated that AHS amendment can increase soil C and N by large amounts, observing a 110% increase in SOC and a 65% increase in Kjeldahl N over ten years. This implies that increases in soil TOC and TN from the AHS amendment may take longer than 210 days to emerge.

Cumulative CO_2

Soil CO_2 evolution is a measure of microbial activity and has long been used as an indicator of soil quality and fertility and a tool for predicting C, N, and P mineralization (Haney et al., 2008). The higher respiration from the GWC soil reflects this basic measure of quality, matching the higher total C and N and the higher total microbial biomass compared to the NC soil. The presence of a carbon source in the form of AHS dramatically elevated microbial respiration, especially where microbial biomass was already higher. However, greater CO_2 evolution may not correlate with an increase in microbial biomass. A meta-analysis (Spohn, 2015) found that microbial respiration both overall and per unit of microbial biomass was higher when the C:N ratio of the litter layer was higher. This agrees with other studies: a trial in an apple orchard recorded greater cumulative CO_2 evolution during a 6-week incubation of the top 6 cm of soil from plots that had received a bark mulch (C:N ratio of 85.3) compared to a chicken manure (C:N ratio of 3.16) (Peck et al., 2011).

Spohn (2015) proposes three explanations for this phenomenon. In N mining, microbes respire the available carbon quickly in order to gain energy for the extraction of recalcitrant N. In overflow respiration, which has been observed in laboratory incubations but plays a questionable role in natural environments, microbes allocate C to respiration simply to dispose of it. Finally, in enzyme inhibition, high N concentrations inhibit enzymes involved in lignin decomposition and slow resource acquisition. Manzoni et al. (2008) discuss the same phenomenon in terms of carbon use efficiency, which they define as the amount of C in new biomass per unit of C decomposed, arguing that decomposers lower their carbon-use efficiency when faced with an N-limited residue. In our study, N mining or overflow respiration could underly the greater CO_2 evolution in response to amendment while minimal impacts were detected on total microbial biomass.

Discussion of CO_2 respired to the atmosphere may beg the question of whether this is a meaningful source of CO_2 emissions in the context of climate change. On average, amended jars lost as CO_2 0.82 g of the carbon applied, or about 7.6%. Some loss to microbial respiration is always necessary to store carbon in soil over the long term, but moisture and application method may influence the amount. A study on straw OMAs performed under laboratory conditions similar to ours—20 °C, 60% relatively humidity, and some treatments kept at a moisture content near field capacity—found that 13% of the carbon from surface-applied straw was lost as CO_2 under continuously moist conditions and only 3% was lost when the soil was allowed to dry to below the permanent wilting point before watering (Curtin et al., 1998). Based on the percentage of amendment mass remaining at the end of our incubation, we estimate the final CO_2 contribution to be only about 20% of the carbon applied when soil is kept near field capacity and lower under typical field conditions. The fact that AHS are surface-applied is also an advantage when considering carbon emissions. Curtin et al. (1998) found that the amount of carbon lost as CO_2 was much higher when straw OMA was incorporated into the soil rather than surface-applied and this effect did not depend on straw type. This supports surface application of OMAs where possible to reduce CO_2 emissions both by reducing machinery use for tillage and by altering microbial activity.

Microbial respiration rates in the amended treatments showed a decline from a high initial rate upon rewetting which was followed by a second peak around 120 days and a second decline. This two-part decomposition pattern could indicate that microorganisms depleted the labile carbon pool and moved on to a more recalcitrant one. Generalizing across a broad range of soil ecosystems, Grandy and Neff (2008) assert that carbohydrates and proteins are selectively degraded from plant residues, leaving behind less labile materials. Many papers have used two-part or double-exponential models to describe microbial mineralization of a labile pool and a recalcitrant pool of a nutrient (Bernal et al., 1998; Deans et al., 1986; Franzluebbers et al., 1995). The multi-stage decomposition process suggested by a two-pool model further characterizes this amendment as a slow-release option for returning nutrients to the soil.

Mineral N

Our results contradict previous findings by Mallory and Griffin (2007), who observed that during a 282-day incubation, there was lower net mineralization of nitrate in soils with a history of OMAs than without. From the perspective of OMA use adoption, our results are promising since they suggest that repeated applications will be beneficial to net nitrogen mineralization rates. To tease out subtle differences, however, more research may be needed: Mallory and Griffin (2007) also saw an interaction effect between soil history and type of new OMA applied, implying that the effect may vary based on the amendment used. Our AHS amendment had a higher C:N ratio than either of theirs (52:1 compared to 31:1 and 7:1) and was applied at a much higher rate: 18,000 kg/ha compared to 100 kg/ha. Differences could also plausibly be attributed to soil characteristics, although the soils in both studies were well-draining loams.

All treatments led to a net mineralization of nitrogen; this cannot properly be called "immobilization" since microbial N uptake was less than gross N mineralization. Estimated mean values of mineral N were above the baseline for all treatments at all time points except the AHS-NC treatment at Day 30. The mineral N values observed here were comparable to what might be seen in working almond orchards, which can range from 0-50 mg per kg dry soil (Schellenberg et al., 2012). While much of the net N mineralization in our incubation can be attributed to soil organic N pools rather than added nutrients, it is relevant that the scale of measurement is comparable to a field setting. In our field trial, soil mineral N was not measured but leaf nutrient analysis of trees found all were in the adequate range for nitrogen, with no difference among treatments.

Initially, we had hypothesized that a larger starting population might lead to a greater increase in soil microbial biomass, as modeled by an exponential growth equation with a higher initial value. However, our mineral nitrogen findings might instead suggest that there was more population growth where microbial populations were smaller to begin with, temporarily locking up a greater proportion of the N in the amendment. In this alternate hypothesis, a soil microbiome that is underfed may exhibit rapid nitrogen uptake when nutrients become available, while a robust microbial population may follow a different nutrient utilization strategy. We were unable to see any confirmation of this pattern in our microbial biomass data because of the challenges with that data set. Nonetheless, support for the differing nutrient utilization strategies hypothesis might be seen in the microbial respiration data: recall that the AHS amendment more than tripled cumulative CO_2 evolution on the GWC soil but more than quadrupled it, although lower overall, on the NC soil.

Dissolved Organic Carbon

Our DOC results align with previous research, emphasizing the higher carbon release potential of soil C stores built up by a history of OMAs. The fact that soil management history had a greater effect on DOC than even a new carbon source speaks to the importance of making soil management decisions with the long-term impacts in mind.

Amendment Layer

We hypothesize that the increase in amendment residue TN is due to microbial biomass growth in the amendment layer itself, an overlooked and understudied aspect of surface-applied OMAs. In the straw OMA incubation study mentioned earlier, researchers also noted small increases in straw N and attributed this to microbial biomass (Curtin et al., 1998). Since nitrogen is a smaller percentage of the amendment material than carbon, changes may be more apparent as a proportion of the TN pool. Nitrogen accumulation in the litter layer has been observed in forests and other non-agronomic systems, where accumulation is believed to reach a variable critical value before flipping to mineralization (Aber & Melillo, 1982; Manzoni et al., 2008).

Prescott and Vesterdal (2021) emphasize that litter residue in forest ecosystems is always a combination of both plant material and microbial transformation products.

The idea that the amendment litter layer is home to an extended pool of microbial biomass is supported by anecdotal evidence from our field trial. A single composite sample containing decomposing amendment material from each of the four blocks was sent to Ward Laboratories (Kearney, Nebraska) for PLFA analysis in April 2021. Total microbial biomass was 10.75 micrograms per gram of dry residual amendment, which is relatively high. While this evidence is anecdotal, it suggests that microbial biomass within the amendment layer is a significant nutrient pool. Soil PLFA data as well as repeat sampling of the amendment with an adequate number of replicates will be reported in future work from our lab group.

Microbial Biomass

The relationship between microbial biomass and other metrics of N mineralization and immobilization potential is not always straightforward. In one incubation of plant material incorporated into soils with differing starting values of microbial biomass carbon, for instance, higher microbial biomass led to faster mineralization initially but no increase in cumulative net mineralization, as the lower-biomass treatments seemed to catch up over the two-month period (Franzluebber 1995). Bonde et al. (1988) examined the amount of potentially mineralizable nitrogen attributable to microbial biomass and noted that several major studies had yielded conflicting results on the question. The concept of the metabolic quotient, or qCO2, has emerged to describe the rate of respiration per unit of microbial biomass (Anderson & Domsch, 1990) but many different claims have been made about its meaning. Does it indicate carbon use efficiency (Spohn, 2015), ecosystem development and/or disturbance (Wardle & Ghani, 1995), system stress as from pH (Anderson & Domsch, 1993), or the impact of the fungal:bacterial ratio (Sakamoto & Oba, 1994)? Others, like Salazar-Villegas et al. (2016), argue that we should really be using active microbial biomass (AMB) instead of total microbial biomass because of the high levels of microbial dormancy. These ongoing debates underscore the complexity of soil microbial biomass and all that we do not yet know about it.

We were unable to draw strong conclusions about soil microbial biomass in our incubation trail. In our field trial, however, patterns did emerge. We attribute the lower microbial biomass in the AHS treatments at the spring sampling date to cooler temperatures created by the mulching effect, which may be especially important in spring when temperatures are more marginal. By fall, this trend had reversed. Based on the long-term effects of OMAs established in the literature, we predict that the AHS amended soils in our field trial will continue to increase in microbial biomass over time compared to unamended plots. Since catch-frame harvest took place for the first time in late summer, shortly before our one-year sampling date, it is logical to conclude that any effect of harvest strategy will require more time to emerge, but we expect the reduction in soil surface disturbance when replacing traditional harvest with catch-frame harvest to lead to greater soil microbial biomass over time as well. This field trial is set to continue for multiple years and may illuminate both effects further.

Implications and Conclusion

Our findings reaffirm that almond hulls and shells and green waste compost, like other OMAs, stimulate the soil microbiome and add nutrients to the soil ecosystem and that, when these nutrient stores have built up over time, they can have long-lasting effects. Other results are more surprising, reversing previous findings or introducing new questions. Field trials on AHS

OMAs and catch-frame harvest are ongoing and will be published by other lab members, and the field trial discussed here is only one of several in progress to evaluate more sustainable practices in almond agroecosystems.

Our research supports the use of almond hulls and shells as an organic matter amendment in almond orchards. AHS increased microbial respiration (incubation trial) and microbial biomass (field trial). Our characterization of AHS decomposition rates and atmospheric CO_2 contributions can inform the strategic application of this material. We also found that layering various types of OMAs on the same field is likely to have positive results on balance, sometimes providing a synergistic interaction effect and potentially mitigating any N immobilization from subsequent amendment applications. Furthermore, we showed that immobilization is not a substantial problem with AHS OMAs as all treatments displayed net nitrogen mineralization starting early in the incubation and all trees sampled in the field trial had adequate N supply. Finally, we observed a non-significant trend suggesting that catch-frame harvest might allow for greater microbial growth in upper soil horizons by reducing disturbances. All these findings support the move to more sustainable management practices within the almond industry.

Beyond management recommendations, we have reversed previous findings on the question of whether soil amendment history increases or decreases nitrogen immobilization (Mallory & Griffin, 2007) and suggested that the answer to this question may depend on amendment characteristics. We agree with the consensus that these effects are small compared to the scale of most N management decisions. We have also raised the question of how the litter layer microbial biomass pool relates to the below-ground pool when using surface-applied OMAs. Future research could characterize the size and composition of these two communities and to what extent they are related and interconnected, such as, for example, to what extent fungal mycelium and fruiting bodies (mushrooms) we observed on the amendment residue are part of below-ground networks. Finally, our CO_2 evolution data suggests that AHS decomposition generates distinct waves of microbial activity that could be related to the multiple forms of carbon present. Better characterizing this dynamic by using more frequent measurements over a longer timespan that allows for near-complete decomposition would be an interesting case study that would further improve our predictions of decomposition timing and CO_2 emissions.

Final take-aways

Almond hulls and shells are a valuable organic matter amendment that provide needed plant nutrients over a 1–2-year timespan and do not seem to cause problematic N immobilization in orchard settings. They have physical, chemical, and biological benefits demonstrated here and elsewhere (see Chapter 1) that are reminiscent of forest litter layers. Repeated application of this amendment alone or in conjunction with other OMAs, such as green waste compost, is likely to have long-term benefits to the soil microbiome and soil health. This is only one of the ways the economic and environmental viability of almond agroecosystems can be enhanced by emerging practices that pair ancient knowledge with modern microbiome scholarship.

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