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Soil Health Responses to Short- and Long-Term Organic Inputs

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Soil Health Responses to Short- and Long-Term Organic Inputs

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ABSTRACT

Over 400,000 acres of California farmland are certified organic and receive all fertility from organic inputs. In addition, due to new legislation requiring the diversion of organic waste from landfills into composting facilities, compost usage is likely to increase even on non-organically managed land. Organic inputs can potentially have many positive effects on soil health, such as increasing the soil organic carbon (C) content, nitrogen (N) cycling for plant growth, and improving the resilience of soil functions to stressful events. However, they may also have negative effects: they can be expensive, and inappropriate application may lead to poor crop growth or environmental pollution. To manage organic inputs in such a way as to maximize the benefits and reduce the risks, California growers need good region- and system-specific information on the effects of a range of organic inputs on the many facets of soil health. The goal of this study is to provide research-based information about the effect of several organic inputs in irrigated California cropping systems on different aspects of soil health, especially as they relate to soil N cycling, C accumulation, and biological function. A secondary focus is to identify important sources of variability both within those effects and within metrics themselves, as soils, amendments, and cropping systems are all intrinsically variable, and "one-size-fits-all" recommendations may not be appropriate. One goal of applying organic materials is short-term N fertility, but there is uncertainty about how much N mineralization can be expected. I used lab experiments to measure the range and variability of net N mineralization from different organic amendments and compost types in common use in California, and tested how this mineralization is affected by soil management history, and how it can be predicted by simple biochemical measurements. I found that materials had a wide range of N mineralization potentials, from immobilization to almost 100% availability, and that availability was broadly predicted by the amendment C to N ratio. Soil management history did not affect the N release rate. Another potential short-term effect of organic inputs is to buffer the soil community against shocks. My second experiment explored whether a recent compost addition could increase the resilience of microbial C and N cycling functions to a sudden chemical stress, the addition of elemental sulfur. I found that stress greatly reduced most C cycling indicators and inhibited nitrification, but tended to increase N mineralization, particularly as the soils adapted to stress. Legume residues added to the stressed soils stimulated catabolic processes (i.e. respiration and net N mineralization) to a much greater extent than anabolic processes (accumulation

of biomass C), suggesting a reduced efficiency in the stress-adapted community. Compost addition did not affect how C mineralization or microbial C responded to stress. However, it consistently increased N mineralization, particularly when legume residues were added to the stressed soils, suggesting compost facilitated a community shift towards one with a relatively low need for N relative to C. In the third and fourth parts of this work, I explored the potential effects of long-term compost and cover crop incorporation on soil health by doing a full assessment of biological, physical, and chemical soil health indicators in a long-term field research experiment in which corn and processing tomato have been either conventionally or organically farmed for over 25 years. In this two-pronged approach, I firstly characterized the sensitivity of the different soil health indicators to management, as well as how they vary with growing season, crop type, and sampling date. In the second part, I assessed the management effects on three essential soil functions: C storage, N mineralization and crop yields. I assessed which indicators are most closely and consistently related to these functions across years and crop types. Finally, I used structural equation modeling to test the hypothesis that healthy soils lead to less stressed crops and thus to higher yields. This study found that organic inputs had strongly increased organic matter and biological activity at these sites. Indicators of C storage and especially biological activity tended to fluctuate between years and sampling dates, but were generally unaffected by crop phase. Different indicators were appropriate for predicting C storage and N mineralization, and I concluded that a holistic soil health assessment should include both indicator types. However, since yields were lower in the organic than conventional system for both crops, my hypothesis that healthier soils would produce healthier crops was not supported. The results of the path analysis suggested the presence of an unmeasured factor strongly related to both management and yields. In the years of this study this factor was likely disease pressures which built up over the years in the organic system. Together, the data from these experiments will be useful to growers, researchers, and policymakers wishing to assess the potential benefits, limitations and risks of different organic amendments over short and long time scales.

Chapter 1: Nitrogen Mineralization from Organic Amendments Is Variable but Predictable[1]

ABSTRACT

To manage nitrogen (N) efficiently, organic growers must be able to predict the amount and timing of plant-available N from organic amendments. In this study, we measured N mineralization from a variety of organic amendments, including composted animal manures and plant material, pelleted and granular organic fertilizer formulations, slaughter waste products, and hydrolyzed liquid fertilizers. In a laboratory incubation, we measured net N mineralization from materials mixed with either organically or conventionally managed soil at 23°C and 60% water holding capacity after 0, 7, 21, 42, and 84 d. We found that net mineral N change in the amended soils after 84 d of incubation fell into four categories: immobilization to 5% mineralization of applied N for yard trimmings composts, mineralization of 15 to 30% for poultry manure composts, 35 to 55% for granular fertilizers, and 60 to 90% for quick release products. However, across all amendments the amount of plant-available N after 84 d of incubation was well correlated with the carbon (C)/N ratio (R^2 = 0.92). Within amendment types, the C/N ratio predicted N mineralization for yard trimmings composts (R^2 = 0.91), manure composts (R^2 = 0.81), and specialty fertilizer and slaughter products (R^2 = 0.88) but not liquid products (R^2 = 0.11). Soil management history did not consistently affect net N mineralization but may have influenced timing.

1.1 INTRODUCTION

Due to concerns about nitrate (NO_3) leaching into the groundwater, California growers are under increasing legislative pressure to match their N applications with crop demand. Among those are certified organic growers, who occupy over 400,000 ha of California farmland (USDA–NASS, 2017). Synchronizing timing of plant-available N with crop demand is especially challenging for organic growers, who use a wide variety of fertility sources from which N must be mineralized before it is plant available. These sources range from composted municipal yard trimmings to patented pelleted and liquid fertilizer formulations. Observing the “4Rs” of efficient nutrient management (right rate, right time, right type, and right placement) and managing irrigation practices appropriately for such a range of different materials

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requires good information about (i) how much of the amendment N is likely to become available to the current crop, (ii) the predicted timing of N mineralization, and (iii) how environmental and management factors affect amendment N mineralization dynamics. New formulations are continually developed; in particular, specialized pelleted and granular blends are increasingly popular. Little research has been done on these materials, in which many different types of organic materials are combined and processed according to proprietary recipes. In addition, compost properties vary depending on the composting process, which differs among facilities, and the feedstock quality and pile age, which may differ from batch to batch within a facility. Therefore, it would be useful to identify classes of materials that behave in similar ways or easily measurable characteristics that can predict an amendment's N mineralization behavior.

Results from prior studies show that N mineralization dynamics are complex and can be highly variable. A first-order kinetics model is often used to describe decomposition and N mineralization from composted amendments (Bernal et al., 1998a; Hadas and Portnoy, 1994; Hadas et al., 1996) or from both fresh and composted amendments (Agehara and Warncke, 2005; Gale et al., 2006). Other researchers have found that the decomposition of incompletely composted or heterogeneous amendments follows a two-pool model (e.g., , linear mineralization during initial decomposition of the easily available material but first-order kinetics thereafter) (Bernal et al., 1998a). In addition, potentially mineralizable N from an amendment class can differ by an order of magnitude. For example, poultry manure compost studies from around the United States report that organic N mineralization plateaued at anywhere from 5% (Hartz et al., 2000; Preusch et al., 2002) to over 40% of the applied N (Gale et al., 2006; Whitmore, 2007).

It is also uncertain whether the mineralization dynamics of an amendment applied to land transitioning to organic management will be the same as it would be applied to land under long-term organic management, where years of organic matter additions have built up a different microbial community (Fauci and Dick, 1994). Some studies found that net N mineralization from different amendments was unaffected by management history (Hadas et al., 1996; Sanchez et al., 2001) and concluded that N mineralization can be considered an intrinsic amendment property. Conversely, other

studies have found that amendment N mineralization was significantly dampened where labile soil organic C (SOC) was high (Mallory and Griffin, 2007; Tyson and Cabrera, 1993). This dampening is attributed to immobilization by the larger and more active microbial community fostered by increased substrate in high-SOC soils (Mallory and Griffin, 2007). Several studies have examined whether simple biochemical properties can predict potential amendment N mineralization. The C/N ratio is one of the most commonly recommended properties, having been found by multiple studies to closely relate to N mineralization from a wide range of organic amendments (Delin et al., 2012; Gale et al., 2006). However, the quality of the C and the initial ammonium (NH_4) concentration can also be predictive, especially for non-composted amendments, because a high concentration of labile C can stimulate microbial biomass growth and immobilization, and a high NH_4 concentration can indicate a less-decomposed material (Agehara and Warncke, 2005; Bernal et al., 1998b; Burger and Venterea, 2008; Calderón et al., 2005). Other measurements found to be correlated with potential N mineralization or immobilization include total N (Hartz et al., 2000) and short-term CO_2 release (Castellanos and Pratt, 1981).

The objectives of this study were (i) to determine potential net N mineralization amounts and timing for a wide range of amendments commonly used on California organic farms, (ii) to examine whether amount and timing of N mineralization differ between two soils with conventional and organic management histories and different SOC levels, and (iii) to identify amendment biochemical characteristics that could be used to predict the behavior of amendments.

1.2 MATERIALS AND METHODS

In 2017 and 2018, a set of controlled 84-d incubations were performed to determine the N mineralization potential of a range of organic amendments in two soils with different management histories. Both soils were mapped as Yolo silt loam (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). The CONV site (38°32'N, 121°47'W) was a conventionally managed research field near the University of California, Davis; the ORG site (38°53'N, 122°14'W) was a commercial field that had been organically managed for over 10 yr. Conventionally and organically managed soils were chosen to determine whether materials would behave similarly on newly transitioning land as on long-term organic land.

Amendment analyses

A total of 22 amendments used by local organic vegetable growers were selected. All materials were approved for use in organic production by the Organic Materials Review Institute. The amendments included yard trimmings–based and manure-based composts, a vermicompost, several commercial granular or pelleted products formulated with manures and slaughter wastes, slaughter products (blood and feather meal), a liquid fish emulsion designed for fertigation, and a hydrolyzed food waste liquid (Table 1.1). Because liquid organic fertilizers have components that settle out, the food-based liquid was incubated both with and without agitation to suspend particles immediately prior to decanting. Compost types included yard trimmings compost (YTC), poultry manure compost (PMC), and compost made from poultry and plant waste (PMC/YTC). The YTCs and PMCs were each obtained from manufacturers in different counties: Yolo and Solano for YTCs (YTC-Y and YTC-S) and Sutter and Merced for PMCs (PMC-S and PMC-M). From the Yolo YTC facility, samples from five batches were collected during 2017 and 2018 and incubated separately (YTC-Y 1 through 5). From the Sutter PMC facility, samples collected from three batches were incubated separately (PMC-S1-3). Descriptions of each amendment are found in Table 1.1. Fresh amendments were stored at 4°C in sealed bags until use.

Moisture content was determined by freeze-drying the liquid amendments and drying the solid amendments at 105°C until weights were stable. Total C and N were analyzed by dry combustion of finely ground samples on a Costech Elemental Analyzer (Costech Analytical) according to Nelson and Sommers (1982). To prevent N loss by ammonia (NH_3) volatilization, materials with a high initial NH_4 concentration were ground and analyzed at ambient moisture when possible or acidified with 3 M HCl and dried at 60°C (Derikx et al., 1994). Amendment C and N concentrations are hereafter reported on a dry-weight basis for all solid amendments and on a fresh-weight basis for liquids (Table 1.1).

Table 1.1 Properties of amendments incubated in spring 2017, fall 2017, and fall 2018

Category	Amendment description	ID	Incubation Date	Moisture %	Total N % †	C:N	$N_{min}0$ % of total N
Plant-based compost	Yard trimmings compost, Yolo (batch 1; mixed with gypsum)	YTC-Y1	Sp2017	59.05	0.72	20.80	0.20
	Yard trimmings compost, Yolo (batch 2)	YTC-Y2	Sp2017	69.29	1.35	18.42	0.45
	Yard trimmings compost, Yolo (batch 3; larger chunks)	YTC-Y3	Sp2017	71.11	1.44	20.14	0.09
	Yard trimmings compost, Yolo (batch 4)	YTC-Y4	Sp2017	57.88	0.89	19.61	0.61
	Yard trimmings compost, Yolo (batch 5)	YTC-Y5	Fa2018	44.64	1.78	13.64	1.27
	Yard trimmings compost, Solano	YTC-S	Fa2017	49.49	1.46	13.22	2.32
Manure-based compost	Yard trimmings/ poultry manure compost blend	YTC/PMC	Fa2018	60.07	2.64	12.05	9.91
	Vermicompost (on cattle manure composted with rice hulls)	Verm	Fa2017	126.80	2.61	13.17	17.77
	Poultry manure compost, Sutter (April 2017 batch)	PMC-S1	Sp2017	46.24	5.27	7.87	15.90
	Poultry manure compost, Sutter (Oct 2017 batch)	PMC-S2	Fa2017	33.84	3.72	6.84	14.77
	Poultry manure compost, Sutter (April 2018 batch)	PMC-S3	Fa2018	41.11	4.69	7.52	16.24
	Poultry manure compost, Merced	PMC-M	Fa2017	43.19	4.27	6.68	25.11
Pelleted/ granular fertilizers	Granular fertilizer, 2% N (poultry manure based)	GF2%	Fa2017	9.36	3.13	6.33	16.84
	Granular fertilizer, 4% N (poultry manure and fish based)	GF4%	ALL	17.14	4.55	6.54	22.75
	Pelleted fertilizer (poultry manure, bone meal based)	PF4%	Sp2017	11.27	4.27	7.34	9.31
	Pelleted fertilizer (poultry manure, feather meal based)	PF6%	Sp2017	10.33	7.13	5.16	3.71
	Pelleted seabird guano	Guano	Sp2017	11.14	12.53	1.15	55.06
Slaughter products	Blood meal	Blood	Fa2018	9.61	13.83	3.51	0.45
	Feather meal	Feather	Fa2017	7.75	15.64	3.83	0.79
Liquid products	Food-based liquid fertilizer, poorly shaken	Food	Fa2017	60.34	2.78	5.16	12.76
	Food-based liquid fertilizer, well shaken	FoodS	Fa2018	60.34	3.13	4.58	11.64
	Liquid fish emulsion	Fish	Fa2017	68.69	2.03	5.19	14.49

† Amendment N concentration expressed on a dry-weight basis for the solid amendments and fresh-weight basis for liquid products

Soil collection and analyses

Amendments were incubated in three separate batches in spring and fall of 2017 and in fall of 2018. Shortly before each incubation, soils were obtained from the top 15 cm of the CONV and ORG sites. Spring samples were taken before planting but after cover crop incorporation in the ORG site and after spring tillage at the CONV site. Fall samples were taken just after the final hand harvest, while the tomato plants were still standing, in fall 2017 at both sites and in fall 2018 at the CONV site. In fall 2018 at the ORG site, soil was sampled after wheat grain had been harvested and the straw removed. Fresh soils were kept at 4°C until use, which was within 8 wk of collection. Although some mineralization occurred during storage, the inclusion of one test amendment (granular fertilizer GF4%) with every incubation ensured that different storage times did not affect the net mineralization from amendments.

Baseline soil properties were measured in spring 2017 on soils collected from the plow layer (top 30 cm) as part of a general site characterization at both the ORG and CONV sites (Table 1.2). Soils were analyzed for moisture by drying in an oven at 105°C for 24 h and for electrical conductivity and pH in a 2:1 deionized water–soil slurry (Smith and Doran, 1996). Soil organic C, texture, and water holding capacity (WHC) were analyzed using dry combustion (Nelson and Sommers, 1996), the pipette method (Gee and Or, 2002), and the funnel method (Wade et al., 2016), respectively. To obtain initial mineral N concentrations of the soil and amendments, fresh ORG or CONV soil (sieved <8 mm) equivalent to 100 g of oven-dry soil was mixed with the equivalent of 336 kg total N ha^{-1} (172 mg N kg^{-1} dry soil) of each amendment. This rate was chosen as the minimum at which all amendments could be uniformly mixed with the soil without pulverization. This is higher than a normal field application rate for most amendments, and at such a high rate some N may be lost by volatilization as NH_3 from the high N amendments. However, the loss is not likely to be substantial: Martin and Chapman (1951) found that 3% or less of the added N was volatilized from 500 mg N kg^{-1} as dried blood mixed with a Yolo sandy loam soil and incubated under similar moisture, temperature, and pH conditions. Additionally, Gale et al. (2006) observed a 1:1 correlation between mineralization potentials from amendments incorporated at rates up to 500 mg N kg^{-1} in a laboratory incubation and the same amendments incorporated at lower rates in the field. Pieces of undecomposed plant matter in the composts weighing more than 10% of the total amount

added were excluded. An unamended control was also included. Two 12-g subsamples were immediately extracted for NH_4–N and NO_3–N analysis as described by Geisseler et al. (2009).

Table 1.2 Initial properties of conventional (CONV) and organic (ORG) soils. Soils were collected from the top 30 cm prior to planting in spring of 2017 or shortly postharvest in fall of 2018. Numbers in parentheses are standard errors of the mean

	CONV	ORG
Location	Davis, CA	Guinda, CA
Soil series	Yolo Silt Loam	Yolo Silt Loam
Rotation (Summer 2016-2018)	Barley/ tomatoes/ sweet corn. Fallow over winter. Conventional fertilizers.	Melons/ tomatoes/ wheat. Oat-legume cover crop over winter. Spring compost addition (withheld Spring 2017).
Sand (%)	21	53
Clay (%)	32	19
EC (2:1 water slurry; mS m^{-1})	8.41 *(0.29)*	18.66 *(0.37)*
pH (2:1 water slurry)	7.75 *(0.02)*	7.08 *(0.01)*
WHC (g g^{-1} dry soil)	0.41 *(0.006)*	0.39 *(0.005)*
SOC (%)	0.88 *(0.006)*	1.21 *(0.035)*
Total N (%)	0.10 *(001)*	0.11 *(0.002)*
C:N ratio	8.94 *(0.04)*	10.86 *(0.12)*

Amendment incubations

Net amendment N mineralization was determined in three incubations, each of which was organized as a randomized complete block design with four replicates. One amendment, a granular fertilizer with 4% N (GF4%), was used in all incubations to ensure comparability. This amendment was reanalyzed immediately prior to each use to ensure no N loss had occurred during storage.

Fresh soil equivalent to 300 g of oven-dry ORG or CONV soil was sieved to 8 mm and thoroughly mixed with an equivalent of 336 kg N ha^{-1} of each amendment by shaking in a large polyethylene bag. Unamended controls were similarly shaken. Pelleted and granular amendments were lightly crushed if necessary for adequate mixing. Soils were transferred to 473-mL plastic cups and packed to a bulk density of 1.3 g cm^{-1}, and moisture was uniformly adjusted to 60% WHC (0.23–0.25 g H_2O g oven-dry $soil^{-1}$) with deionized water using a syringe with a side-port needle. Soils receiving the liquid amendments were mixed and packed as described above and uniformly injected with the equivalent of 336 kg N ha^{-1} of amendment diluted in the water used to adjust the moisture. Cups were covered with perforated plastic

film and kept in loosely covered bins at 23°C. The moisture content was chosen to provide optimum conditions for microbial activity, and the soil temperature is typical for summer-grown irrigated vegetable crops in Yolo County. Incubation studies performed at similar moisture and temperatures, although not necessarily representative of field conditions, have been generally found to be good proxies for mineral fertilizer equivalent in the field or greenhouse (Delin et al., 2012; Gale et al., 2006; Hartz et al., 2000; Spargo et al., 2016).

Samples were periodically aerated by fanning, and moisture was maintained between 50 and 60% WHC. Cups were destructively harvested after 7, 21, 42, and 84 d of incubation, and subsamples were extracted for NH_4–N and NO_3–N analysis as described above.

Modeling and statistical analysis

For each amendment, the following parameters were calculated. The proportion of the total added N (N_{tot}) initially in mineral form ($N_{min}0$) was calculated as:

$$N_{min}0 = \frac{(NH_4 - N + NO_3 - N)_{amended} - (NH_4 - N + NO_3 - N)_{control}}{N_{tot}}$$

for amended and unamended control samples extracted directly after mixing. The proportion N_{tot} in mineral form at time *t* (in days) was calculated as:

$$N_{min}t = \frac{(NH_4 - N + NO_3 - N)_{amended} - (NH_4 - N + NO_3 - N)_{control}}{N_{tot}}$$

The proportion of organic N ($N_{org}t$) mineralized with each amendment at time *t* was calculated as:

$$N_{min}t - N_{min}0$$

The $N_{min}t$ values for each time *t* were compared using PROC MIXED in SAS (SAS Institute) such that each incubation date (spring 2017, fall 2017, and fall 2018) was analyzed as a separate randomized complete block design experiment with replicates as blocks. Means separation was performed with Tukey's test using an α of 0.05. Blocks were treated as random effects; treatment and soil type were fixed effects. One amendment, GF4%, was incubated at each date to ensure conditions were comparable.

Incubation dates were compared by testing the GF4% date and date × soil interactions for the amount of plant-available N after 84 d of incubation ($N_{min}84$) using PROC MIXED in SAS.

Potential net N mineralization of the added organic N (N_0) and rate constant (k) were also calculated for each amendment as described in Appendix 1.

1.3 RESULTS AND DISCUSSION

None of the parameters tested for GF4% differed significantly ($p > 0.10$) among incubation dates for either soil type. Therefore, results were considered to be comparable for amendments incubated in different batches.

Rate and timing of amendment nitrogen availability

The results demonstrate the wide range of N mineralization dynamics and potential crop availability from different amendment types used by California organic growers (Fig. 1.1). Because overall mineralization patterns were very similar between the ORG and CONV soils, amendment curves in Fig. 1.1 represent average values. Full data are given in Appendix 1 Table 1. The tested amendments fell generally into four classes: Class a, YTCs from which <5% of N was in mineral form after 84 d of incubation; Class b, manure composts from which $N_{min}84$ = 15 to 30% of applied N; Class c, granular fertilizers from which $N_{min}84$ = 35 to 55%, most of which was already in mineral form within a few weeks of application; and Class d, quick-release liquids, slaughter products, and guano, from which $N_{min}84$ = 60 to 90%. The PMC/YTC compost and vermicompost fell intermediate of Class a and Class b.

Mineralization patterns followed three different dynamics (Fig. 1.1). In the first pattern, which was observed for the vermicompost and most of the YTCs, mineral N remained fairly stable over 84 d (Fig. 1.1a and 1.1b). The YTCs all started with low N concentrations and over the 84 d either immobilized N or mineralized <5% of their total N. Similarly, Hartz et al. (2000) studied several California municipal yard waste composts and found that between −1.9 and 5.4% of the total N was available after 84 d of incubation at 25°C. The YTCs tested are therefore a negligible source of plant-available N during the growing season in which they are applied. At the measured N concentrations, a YTC application of 22 Mg ha^{-1} would add 100 to 200 kg N ha^{-1} to the soil. Even at the highest measured mineralization rate, only about 5 to 10 kg N ha^{-1} of that would be expected to be mineralized in warm and moist soil over a 3-mo

period. However, over time their application may contribute to long-term soil fertility. In a 7-yr experiment with different green waste composts, Sullivan et al. (2003) found that, in the first year after a large application (155 Mg ha^{-1}), composts either reduced or had no effect on grass yield compared with a no-compost control. Over the following 6 yr, however, grass yield, N uptake, and soil mineral N were increased in the amended plots.

Similar to the YTCs, vermicompost mineral N was relatively stable over the 84 d. However, unlike the YTCs, the vermicompost started with the relatively high $N_{min}0$ of 17.8%, and, unlike all the other tested amendments, the majority of this was in the form of NO_3–N. A low ratio of NH_4–N to NO_3–N is generally considered an indicator of compost stability (Bernal et al., 1998b). The lack of further N mineralization during the incubation also suggests a very stable product. Flavel and Murphy (2006) also report vermicompost to be highly stable.

In the second pattern, which included PMC from the Merced facility (PMC-M), the third PMC batch from the Sutter facility (PMC-S3), and the third YTC batch from the Yolo facility (YTC-Y3), N was initially immobilized and then slowly increased (Fig. 1.1a and 1.1b). The pattern of quick net immobilization followed by gradual net mineralization is characteristic of non- or incompletely composted complex organic substrates (Bernal et al., 1998a; CCQC, 2001). These materials still contain relatively undecomposed and labile C that stimulates soil microbes to immobilize N, which is then slowly re-mineralized as the microbial biomass turns over (Bernal et al., 1998; Burger and Venterea, 2008; Calderón et al., 2005). For YTC-Y3, which had negligible $N_{min}0$, this immobilization caused mineral N to be well below the control ($N_{min}t < 0$) throughout the incubation (Fig. 1.1a). Our results show that PMC from the Merced facility and PMC-S3 had the highest initial NH_4–N concentrations and NH_4/NO_3 ratios and were the only two PMCs to follow this pattern. They also had less additional N mineralization over 84 d compared with PMC-S1 and PMC-S2. Although microbial biomass was not measured, this result is in line with Calderón et al. (2005), who found that the initial NH_4 concentration in manure was positively correlated with microbial N immobilization. Another possible explanation for the decline is volatilization as NH_3, which can occur under high NH_4 concentrations at a high pH (Hadas et al., 1983). However, this is unlikely to have been the major cause because similar declines were observed for both the alkaline CONV soil and the neutral ORG soil (Appendix 1 Table 1).

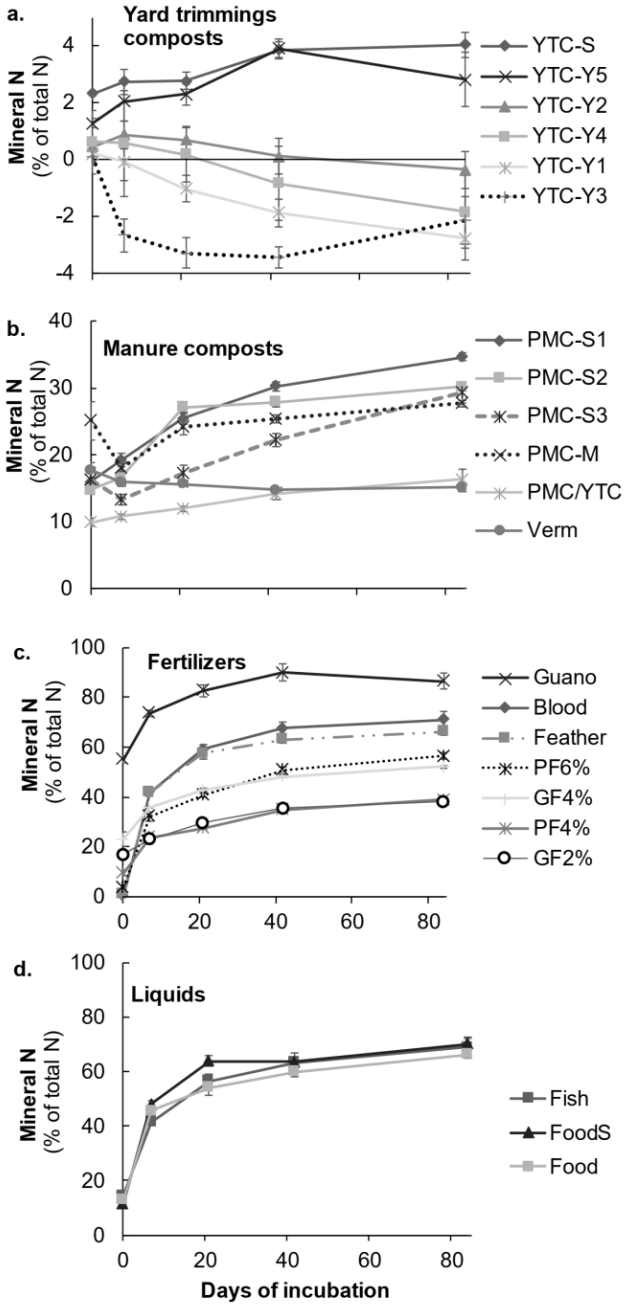

Figure 1.1. Changes in soil mineral N concentrations associated with organic amendments incubated at 23°C for 84 d, with reference to unamended control soils. Values are averages of amendments incubated in conventional (CONV) and organic (ORG) soils. (a) Yard trimmings compost. YTC-Y and YTC-S, yard trimmings compost from Yolo and Solano facilities, respectively. (b) Manure composts. PMC-S and PMC-M, poultry manure composts from Sutter and Merced facilities, respectively; PMC/YTC, blend of poultry and yard wastes; Verm, vermicompost. (c) Fertilizers. Blood and Feather, blood and feather meal, respectively; GF, granular fertilizer; PF, pelleted fertilizer. (d) Liquids. Fish, fish emulsion; FoodS and Food, shaken and unshaken food hydrolysate, respectively. Bars represent SEM (n = 4).

In the third pattern, N mineralization followed first-order kinetics. This pattern was observed for pelleted/granular, slaughter, and liquid products as well as PMC-S1, PMC-S2, and to some extent PMC/YTC. In these amendments, N was rapidly mineralized during the first few weeks. Mineralization slowed after between 21 and 42 d and tended to plateau thereafter. Within this broad pattern, the $N_{min}0$ and the proportion of N mineralized varied widely for different materials. Average $N_{min}84$ from amendments following first-order kinetics ranged from 38 to 87% of added N, with higher proportions mineralized from low C/N ratio amendments (see below). Average guano $N_{min}0$ was 55%. The guano $N_{min}0$ in this study was high compared with values observed by other studies, which range from 5 to about 20% (Hadas and Rosenberg, 1992; Hartz and Johnstone, 2006; Manojlović et al., 2010); however, the potential plant-available N in those studies was similar to observed $N_{min}84$ values. In contrast, the slaughter products' $N_{min}0$ was <1%, but 40% of their N was mineralized within 7 d. The N in these products consists almost entirely of protein, which is hydrolyzed by proteases when incorporated into soil (Ciavatta et al., 1997; Hadas and Kautsky, 1994; Jan et al., 2009). The potentially plant-available N observed from slaughter and liquid amendments was similar to that observed in other studies (Delin et al., 2012; Hadas and Kautsky, 1994; Hadas and Rosenberg, 1992; Hartz and Johnstone, 2006; Hartz et al., 2010; Manojlović et al., 2010), which found that mineral N plateaued at 60 to 80% of added N. Most of this was mineralized within the first 2 wk. Curves for the pelleted and granular blends tended to resemble combinations of the amendments from which they were made, suggesting that processing did not notably change their release properties.

The N mineralization rates of unshaken food hydrolysate (Food) and shaken food hydrolysate (FoodS) were not compared statistically because they were incubated at different dates; however, FoodS appeared to be slightly faster (Fig. 1.1d), suggesting a greater lability of the components that had fallen out of suspension. The N_{tot} of FoodS was 12% higher than that of Food (Table 1.1). Similarly, Hartz et al. (2010) found that about 8 to 21% of N contained in liquid fertilizers resided in particulate materials, which may be lost during filtration. Although values for $N_{min}84$ were similar between FoodS and Food, FoodS had greater N_{tot}, indicating a higher absolute net mineral N concentration after 84 d of incubation.

Despite different mineralization dynamics, $N_{min}84$ values were similar among PMCs from different batches and facilities. However, comparison with other studies suggests the material is more variable.

The composition of a PMC varies depending on type of poultry, composting method and duration, bedding material, and storage method (Bernal et al., 1998a; Gale et al., 2006; Leconte et al., 2011; Preusch et al., 2002; Tyson and Cabrera, 1993). Both facilities in this study used rice hulls as the bedding material, which is a common agricultural waste in California but not in most of the United States. The $N_{min}0$ values in these PMCs are greater than those reported by Hartz et al. (2000), who collected seven PMCs from around California and found initial inorganic N concentrations ranging from 0 to 8% of the amendment's total N, with an additional 3 to 15% of the organic N mineralized over 84 d of incubation at 25°C. Other studies with PMCs have found $N_{min}0$ values of <5%, with additional mineralization potentials of <10% (Leconte et al., 2011; Preusch et al., 2002; Tyson and Cabrera, 1993). In contrast, $N_{min}0$ from PMCs in the current study ranged from 15 to 25% of their total N content (Table 1.1), and N_0 plateaued at around 20% of organic N (Appendix 1 Table 2). The $N_{min}84$ values were similar to potentially plant-available N measured in uncomposted or incompletely composted poultry litters (Gale et al., 2006; Sims, 1986). As discussed above, it is possible that the PMCs in our study had not completed the composting process.

The N mineralization amount and timing reported here are more accurate for warm and moist soils; mineralization may be slower in drier soils or under cool conditions. Where N release follows first-order kinetics, temperature affects the rate constant k more than the mineralization potential N_0 (De Neve et al., 1996; Griffin and Honeycutt, 2000). For high-N materials, temperature differences would mainly be important during the first few days after incorporation, and therefore differences may not be on a scale relevant to growers. Hartz and Johnstone (2006) observed that, for four high-N amendments at 4 wk of incubation, a temperature decrease from 25 to 10°C decreased mineralization by 20% or less. A similar effect was observed for liquid amendments incubated at 15 and 25°C (Hartz et al., 2010). For low-N materials and those that do not follow first-order kinetics, the mineralization potential is more likely to be affected for a longer period (Agehara and Warncke, 2005).

Soil effect on amendment nitrogen mineralization

Net N mineralization was higher in the unamended ORG soils than in the CONV soils at all dates (Fig. 1.2). This is unsurprising given the ORG site's long-term history of compost and cover crop addition; samples taken in spring from the plow layer of both sites show that SOC was 38% greater in the ORG soil

than in the CONV soil (Table 1.2). For the CONV site, which received neither winter cover crop nor compost, N mineralization was similar for soils collected at all dates. Conversely, the ORG soils mineralized more N in spring than at either fall date, suggesting that the recently incorporated cover crop, which was the only amendment applied to the ORG soil in that year, contributed a large pool of labile organic matter relative to the fall soils. These were collected at the end of the season and had received no residue inputs (i.e., tomato plants were still standing in fall 2017 and fall 2018 CONV soil, and in fall 2018 ORG soil wheat straw had been removed).

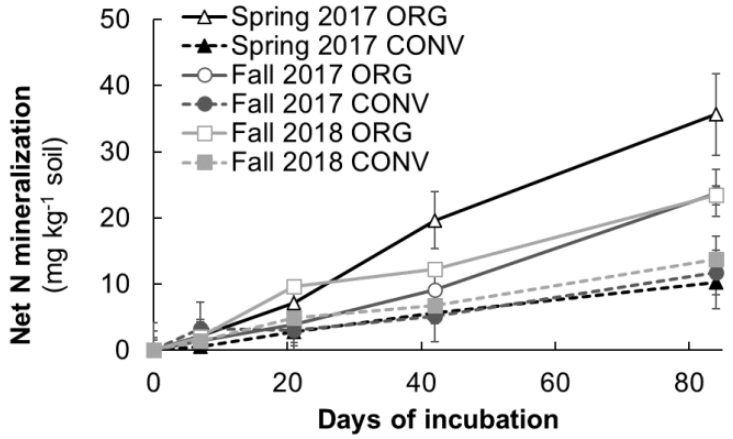

Figure 1.2. Nitrogen mineralized from organic (ORG) and conventional (CONV) control soils over 84 d of incubation at 23°C and 60% water holding capacity. Soils were collected from the same fields in spring and fall of 2017 and fall of 2018. Bars represent SEM (n = 4).

Table 1.3 Significance of the simple and interactive effects of amendment type and soil on available N after 7 ($N_{min}7$), 21 ($N_{min}21$), 42 ($N_{min}42$), and 84 ($N_{min}84$) days of incubation at 23°C and 60% water holding capacity. Comparisons were only made within an incubation date.

		Measured available N after 7, 21, 42 and 84 days			
Date	Effect	$N_{min}7$	$N_{min}21$	$N_{min}42$	$N_{min}84$
Spring 2017	Amend	<.0001	<.0001	<.0001	<.0001
	Soil	0.0007	0.4601	0.0765	0.5744
	Amend*soil	0.4419	0.9936	0.1403	0.1746
Fall 2017	Amend	<.0001	<.0001	<.0001	<.0001
	Soil	0.4132	0.9625	0.7287	0.1207
	Amend*soil	0.5909	0.5217	0.8427	0.7164
Fall 2018	Amend	<.0001	<.0001	<.0001	<.0001
	Soil	0.8224	0.8055	0.0137	0.0627
	Amend*soil	0.0967	0.4264	0.0276	0.1131

Despite the soil differences, $N_{min}84$ did not differ significantly between the two soil types for any of the amendments (Table 1.3). A significant soil effect was observed during the first week of the spring incubation, where available N after 7 d of incubation in the CONV soils was significantly higher than in the ORG soils (Table 1.3; Appendix 1 Table 1). At that date, all ORG amendments had lower available N after 7 d of incubation than their CONV counterparts (Appendix 1 Table 1), suggesting that the significant soil effects were similar across all amendments. A significant main effect of soil and interaction with amendment was observed at 42 d of incubation in the Fall 2018 incubation (Table 1.3). Two amendments (the GF4% and YTC/PMC blend) had lower available N after 42 d of incubation in the CONV soil than in the ORG soil at this date. We do not have a plausible explanation for this temporary difference.

Our results are in line with many studies that observed that soil management history has only a transitory effect on net N mineralization from recently added amendments (Hadas et al., 1996; Nett et al., 2012; Stark et al., 2008). However, the difference in the first week of the spring incubation has implications for the dynamics of N release. Modeling the rate constant and mineralization potential for amendments that followed first-order kinetics shows a higher mineralization rate constant but a lower mineralization potential for the CONV soil in spring—that is, in the spring CONV soil amendment N mineralized more quickly than in the ORG soil but plateaued at a lower value (Appendix 1 Table 2). The temporary dampening of net N mineralization from amendments incubated in the ORG soil in spring is consistent with the observation that, when a soil has a high concentration of readily available C, more of its N will be immobilized into the microbial biomass, from which it is later slowly mineralized (Burger and Venterea, 2008; Mallory and Griffin, 2007).

Although the two soils were mapped as the same series, the ORG soil had a sandier texture than the CONV soil, such that management and texture were confounded (Table 1.1). Several studies have observed greater net mineralization from coarse-textured soils than from fine-textured soils (Castellanos and Pratt, 1981; Gordillo and Cabrera, 1997; Sørensen and Jensen, 1995), an effect often attributed to increased microbial biomass in higher-clay soils due to a more protected habitat (Amato and Ladd, 1992). However, the fact that differences between ORG and CONV soils occurred in spring but not fall suggests they were related to management more than to texture. Additionally, in this case it was the finer-textured CONV soil that had temporarily higher mineralization.

Amendment biochemical characteristics

The N concentrations in the incubated amendments ranged from 0.72 to 15.6% of their dry weight (Table 1.1). The C/N ratios ranged from a low of near 1:1 in the pelleted seabird guano to over 20:1 in some batches of YTC-Y. As expected, the manure composts as well as the pelleted, slaughter, and liquid amendments had higher N concentrations than the plant-based composts. Expressed as they are marketed (i.e., on a liquid basis), the 2 to 3% N concentrations for the liquid amendments were low compared with other fertilizers designed as sources of quickly available N to growing plants. When calculated on a dry-weight basis, the values were more comparable (6–8% N). The C/N ratios for the liquid amendments were similar to those of higher-N amendments (on average around 5:1).

Across the dataset, the C/N ratio was a good indicator of an amendment's $N_{min}84$ (Fig. 1.3a). The $N_{min}84$ was also strongly related to the N concentration when the latter was expressed on a dry-weight basis for all amendments (Fig. 1.3b). This relationship broke down if the fresh weight N concentration of the liquid amendments was used (R^2 = 0.46). Liquid amendment N concentrations are always guaranteed and are reported on a fresh-weight basis; therefore, the fact that the C/N ratio is independent of the amendment's moisture content makes it a more useful parameter for comparing liquid and solid amendments. The C/N ratio was a better predictor of $N_{min}84$ than N concentration for the fertilizers (pelleted and slaughter products; R^2 = 0.88 and 0.70, respectively) and YTCs (R^2 = 0.90 and 0.51, respectively). The N concentration, however, was a better predictor for the manure composts (R^2 = 0.81 and 0.92, respectively). Neither parameter was a good predictor of $N_{min}84$ within the liquid products (R^2 = 0.11 and 0.05, respectively).

The shape of the observed relationship between C/N ratio and mineralized N agreed well with those observed by Gale et al. (2006) and Delin et al. (2012). Both of these studies measured a wide range of composted, noncomposted, and specialty amendments; however, the former study reports mineralization in terms of plant-available N from field-applied amendments, and the latter reports mineralization in terms of mineral fertilizer equivalent in a pot study. In both these studies, the predicted potential N release tended to be slightly (on average around 7%) higher than the $N_{min}84$ we measured at equivalent C/N ratios. This may be due to a variety of factors, including the presence of living plants;

differences in application method, rate, experiment duration, and conditions; and method of calculating N availability.

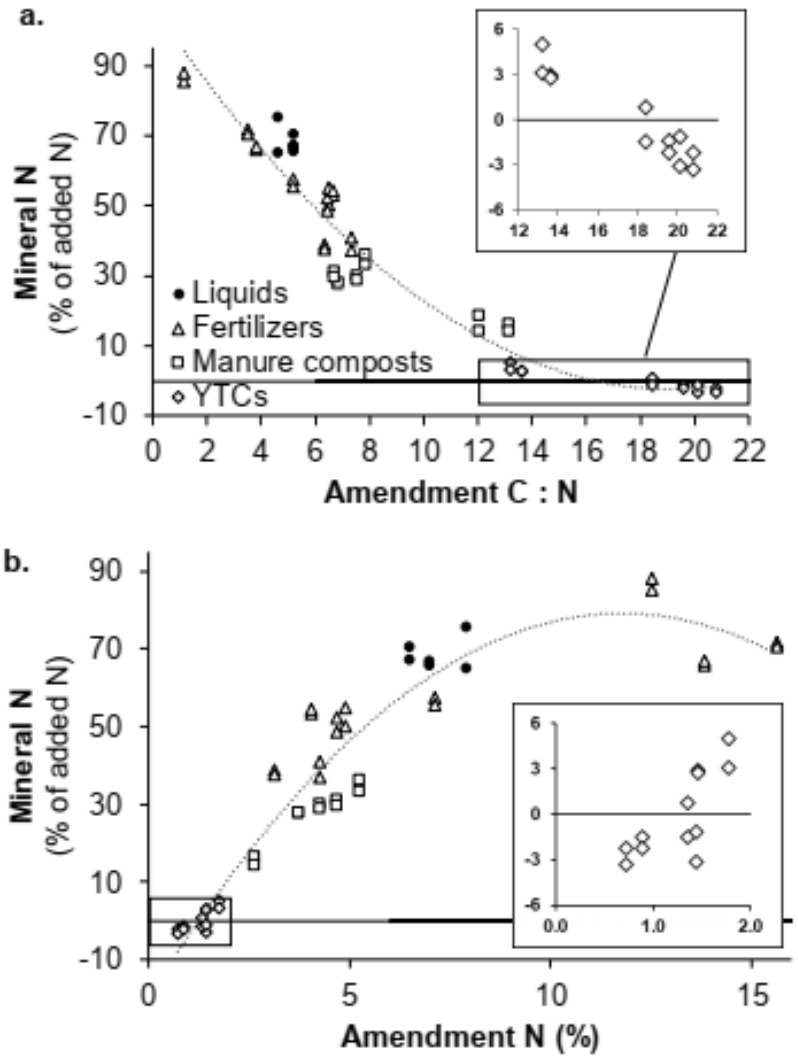

Figure 1.3. Relationship between the proportion of N in the mineral form after 84 d of incubation at 23°C in conventional (CONV) or organic (ORG) soil and (a) amendment C/N ratio and (b) amendment N concentration on a dry-weight basis. Insets represent the yard trimmings composts (YTCs). The R^2 value for the trendline in graph (a) is 0.927. The trendline in graph (b) has an R^2 value of 0.915.

These overall relationships of N availability with C/N ratio or N concentration are more useful for obtaining a general estimate of an amendment's N mineralization potential than for predicting exact

values within a group of similar amendments. This is because the R^2 value is partly a function of the range of C/N ratios or N concentrations under consideration (Hartz et al., 2000). For example, the good correlation for the manure composts is due to the inclusion of the vermicompost and the YTC/PMC blend, which had C/N ratios nearly double those of the PMCs. Within the PMCs, which occurred over a narrow range of C/N ratios, there was no relationship with $N_{min}84$.

The C/N ratio was a more reliable predictor than the N concentration of whether a compost would immobilize N. Several studies have reported organic amendment C/N ratio threshold values above which N is immobilized, including 15:1 (Gale et al., 2006), between 16:1 and 19:1 (Calderón et al., 2005), and 21:1 (van Kessel et al., 2000). In the present study, amendments with C/N ratios >19:1 always immobilized N, whereas amendments with C/N ratios <14:1 always mineralized N. All YTCs with a total N concentration <1.3% (dry weight) immobilized N. However, some of the strongest immobilization occurred in YTC-Y3, which had a relatively high N concentration (1.4% dry weight) but was less decomposed.

No raw manures were included in this study because they are rarely applied to organic vegetables in California due to food safety concerns. With these materials, the initial quality of the C is extremely variable and is likely to have more of an effect on N mineralization potential than in composted amendments, and thus the C/N ratio may be a less reliable indicator (Calderón et al., 2005; Sims, 1986). Composting reduces some of this variability (Preusch et al., 2002).

Implications for 4R management of organic fertilizers

The results of this study have implications for efficient rate, type, timing, and placement of organic fertilizers. The amount of N that will become available from a given fertilizer application rate can be broadly predicted by amendment type and C/N ratio, regardless of soil management. In addition, low rates of guano, liquids, and slaughter products are likely more efficient than high rates, especially on alkaline soils, because amendments that mineralize N quickly can increase salinity and NH_3 concentrations, reducing microbial activity and decreasing the total proportion of N mineralized (Cayuela et al., 2009; Hadas et al., 1983).

Because of compost's slow mineralization, the annual N applications may far exceed plant uptake, raising concerns about potential groundwater pollution if unused N, building up over time, is

mineralized and leached when no crop is present. Although the experiment was not designed to assess this issue, long-term field research suggests that, over time, the N mineralization rate from YTCs remains low enough (<2.5% per year after initial application) that it is unlikely to be a serious risk, especially if cropped year-round (Horrocks et al., 2016; Sullivan et al., 2003). A greater proportion of manure-based compost N becomes plant available, and buildup from annual applications may result in leaching in the absence of winter crops (Evanylo et al., 2008). Excess P accumulation is also a risk from these materials.

Efficient timing for applying organic amendments depends on amendment type. Amendments varied in their degrees of maturity among compost batches and facilities, and immature composts may cause N or oxygen limitations for growing seedlings (Bernal et al., 2009). Individual batches therefore should be tested for maturity (CCQC, 2001), and incorporating immature composts less than a week before planting may be risky. For yard trimmings composts, for which N mineralization is likely slow enough that significant N leaching over winter is less of a risk, application during the previous fall for spring-planted crops may be safest. Conversely, the quick mineralization from almost all the fertilizer products suggests that, under warm and moist conditions, applications should be made as close as possible to the time of plant demand to avoid leaching losses. Preliminary work by our laboratory and studies with similar amendments suggest that significant mineralization can occur within a few weeks even under cooler temperatures (<5°C) (Agehara and Warncke, 2005; Hartz et al., 2010; Sims, 1986).

Initial NH_4 concentration and N mineralization rate have implications for amendment placement. A considerable amount of N may be lost through NH_3 volatilization when amendments with a high NH_4 concentration are surface applied. In manure-based composts, liquid fertilizers, and most granular and pelleted fertilizers, at least 10% of the total N was NH_4–N and would be susceptible to volatilization if not incorporated within a few days (Derikx et al., 1994; Hadas et al., 1983). Concentrated bands of fast-releasing materials such as the guano or slaughter products should be applied at a safe distance from the seedling because fast mineralization rates are associated with high NH_3 concentrations, which may inhibit germination or injure seedlings of sensitive species such as tomato (Diaz-Perez et al., 2017). The low concentrations applied through fertigation, typically 10 to 20 kg N ha^{-1} in an application, are less likely to be a risk.

1.4 CONCLUSIONS

The results of this study can inform the 4Rs of efficient N management for organic fertilizers (right rate, right timing, right type, and right placement). The organic amendments tested have a wide range of potentially crop-available N ranging from immobilization by some yard trimmings composts to 80 to 90% of the N applied as seabird guano. However, across all materials the proportion of total N that was in the mineral form after 84 d of incubation was closely related to the C/N ratio. The timing of potential N mineralization may have been somewhat slowed by the high concentration of labile C present in the organically managed soil in spring, but otherwise N mineralization from all amendments was generally similar between two soils with different textures and management histories.

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Chapter 2: Acid Stress and Compost Differentially Affect Microbial Carbon and Nitrogen Cycling Functions in an Agricultural Soil[2]

ABSTRACT

Agricultural practices can lead to strong fluctuations in soil pH and salinity, likely affecting soil microbial functions. As microbial communities tend to recover more quickly in soils with higher carbon (C) availability, compost addition may reduce the impact of these stresses, potentially leading to more stable and resilient systems. We examined how microbial C and nitrogen (N) cycling functions responded to the imposition and relief of sulfur-induced stress, and whether these responses were moderated by the addition of compost. In a greenhouse pot study, we mixed soil with elemental sulfur and green waste compost in a complete 2-way factorial design. Sulfur induced a strong acidity and mild salinity stress. After 70 d, stress was partially alleviated by leaching with liquid lime. Prior to the stress, after 21 and 42 d of stress, and one week after stress alleviation we performed several C and N cycle assays with and without the addition of ground legume residues to stimulate mineralization and microbial growth responses. Stress greatly reduced most C cycling indicators and inhibited nitrification, but tended to increase N mineralization, particularly as the soils adapted to stress. Legume residues stimulated catabolic processes (i.e. respiration and net N mineralization) to a much greater extent than anabolic processes (accumulation of biomass C), suggesting a reduced efficiency in the stress-adapted community. Compost addition did not affect how C mineralization or microbial C responded to stress. However, it consistently increased N mineralization, particularly when legume residues were added to the stressed soils, suggesting compost facilitated a community shift towards one with a relatively low need for N relative to C.

[2] A version of this chapter has been submitted for publication as “Acid stress and compost differentially affect microbial carbon and nitrogen cycling functions in an agricultural soil” to the journal Biology and Fertility of Soils, and was under review at the time of writing.

2.1 INTRODUCTION

Soil microorganisms drive many soil functions, including the decomposition and transformation of organic material. For those functions to continue, microbes must continually adapt to changing soil conditions, including acidity and salinity. A large body of work from long-term experimental agricultural gradients and across natural landscapes has found that both acidity and salinity can have profound stressful effects on microbial diversity, biomass, growth, activity, efficiency, nutrient cycling functions, and ultimately soil carbon (C) storage potential (i.e. Aciego Pietri and Brookes, 2008b; Kemmitt et al., 2006; Malik et al., 2018; Rath et al., 2019a, 2019b; Rietz and Haynes, 2003; Rousk et al., 2009, 2010a; Silva-Sánchez et al., 2019). In intensive agriculture, irrigation and fertilization can lead to significant and potentially rapid increases in soil salinity and acidity (Haynes and Swift, 1987; Chung and Zazoski, 1993; Hanson and May, 2011; Venterea and Rolston, 2000). Practices such as drip irrigation or fertilizer banding, which aim to improve input use efficiency by targeting water or nutrients to the plant rooting zone, may exacerbate the magnitude and heterogeneity of acidity or salinity stresses by concentrating them in smaller soil volumes (Hanson and May, 2011; Haynes and Swift, 1987). Managed systems also allow these stresses to be rapidly corrected through leaching or liming. While it is likely that these sudden and often combined stresses affect microbially mediated soil functions, almost all of the work examining the impact of acidity and salinity on microbial function has been done in stable natural ecosystems or long-term agricultural gradients in which soils and microbial communities have had decades or centuries to differentiate. Few studies have tested how acidity and salinity impact microbial function under the fluctuating conditions which characterize intensive agriculture and the degree to which those functions can recover after the stress is relieved (Yan and Marscher, 2012).

The ecological concepts of resistance and resilience are particularly applicable to the study of how microbial communities respond to fluctuating stresses. While these terms are defined in many ways, resistance can be thought of as the degree to which a community retains its structure and function during a disturbance and resilience the degree to which it is able to recover to its unstressed condition (Holling, 1973; Shade et al., 2012). On the level of individual cells, important mechanisms include dormancy or stress-induced physiological changes, such as accumulation of osmoprotectants, changes in membrane composition, and the production of specialized enzymes, proteins, organic acids and extracellular

polymeric substances (Auger et al., 2013; Draghi et al., 2016; Chang and Cronan, 2009; Kakumanu and Williams, 2014; Lund et al., 2014; Schimel et al., 2007; Shade et al., 2012; Zhang and Rock, 2008). These mechanisms tend to be particularly important in for short-term stresses. On a population and community level, more adapted taxa or individuals with beneficial mutations grow preferentially, over time potentially leading to community shifts (Rousk et al., 2010a; Shade et al., 2012; Silva-Sánchez et al., 2019).

These mechanisms have two important implications for functional resilience in stressed agricultural soils. Assuming some organisms are able to survive and grow under stressful conditions and others are not, a "broad" function which is performed by a very large range of taxa is likely to be more resilient than a "narrow" one which is only performed by a few specialist organisms, which may or may not be among the survivors (Schimel, 1995). Secondly, cell-level adaptations are energetically expensive and involve tradeoffs with cell biosynthesis and preferential growth occurs more quickly when a C source is available (Draghi et al., 2016; Malik et al., 2018 and 2019; Schimel et al., 2007; Oren, 2008), suggesting both functional recovery and community shift would occur more quickly in the presence of available C substrate (Rath et al., 2019a). In fact, studies have frequently found that the effects of both acidity and salinity on microbial community structure and function, and the length of their recovery time, if the stress is relieved, tend to be comparatively lessened in soils with a higher soil organic matter (SOM) content or where a substrate is added (Aciego Pietri and Brookes, 2009; Rousk et al., 2009, 2010b; Rath et al., 2019b; Wichern et al., 2006). It therefore seems likely that agricultural practices which increase available C, such as compost addition, would buffer the effects of a stressful change on microbial community functions and allow for a faster recovery. However, we are not aware of any studies which test this hypothesis.

To help improve our understanding of microbial responses to stresses occurring in intensively managed systems, and the potential role of compost in moderating those responses, we performed a pot experiment in which acidity and salinity stresses were imposed by mixing powdered elemental sulfur (S) into soil with or without compost. Sulfur was chosen as a stressor because it has a variety of agricultural uses, can be uniformly mixed with soil, and rapidly increases both acidity and, in the absence of leaching,

salinity (McTee et al., 2017; Wiedenfeld, 2011). The first objective of this study was to measure how the microbial biomass and several metrics of C and nitrogen (N) cycling activity are affected by the imposition, persistence, and partial alleviation of acidity and salinity stresses in combination. The second objective was to test whether compost application at an agronomically realistic rate moderates these effects.

We hypothesized that 1) Microbial biomass and metrics of microbial C and N cycling activity would initially be strongly repressed by S-induced stress 2) Partial functional recovery would occur as communities adapt to the stress, with C and N mineralization functions recovering more quickly than nitrification 3) All functions and pools would recover (become more similar to the unstressed control) to varying extents following alleviation, and 4) The presence of compost would buffer the stress response and improve recovery. The present study is part of a larger project that investigates the effects of induced stress and soil health promoting practices on soil microorganisms and crops.

2.2 MATERIALS AND METHODS

Compost and soil collection

Green waste compost was collected from a local commercial composting facility. Compost was thoroughly mixed in a large bin and then stored moist at 4°C in covered buckets until use. Moisture was measured by drying at 105°C for 24 h. Total C and N were measured on dried, ground material by dry combustion (Nelson and Sommers, 1996) on an elemental analyzer (Costech Analytical Technologies, Valencia, California, USA). The compost had a dry matter (DM) concentration of 48%, total N and C content of 15 g kg^{-1} DM and 250 g kg^{-1} DM respectively, and a C:N ratio of 17:1. The electrical conductivity (EC) and pH, as determined in a 5:1 water:compost slurry (Thomas, 1996) were 99 µS cm^{-1} and 8.5.

Soil was collected in early spring from the top 30 cm of an agricultural field near Davis, California (38°32' N, 121°46 W) which had been fallow for at least two years. A neutral, non-saline soil with minimal recent C or fertilizer inputs was chosen to maximize response to compost and S treatments. The soil was mapped as a Yolo Silt Loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Moisture was determined on several subsamples and soils were stored moist in covered bins until use.

Greenhouse experiment

A greenhouse experiment was set up as a randomized complete block design with 2 factors and 5 replicate blocks. All soil for each replicate was sieved using 12 mm mesh and thoroughly homogenized prior to applying the treatments. For each pot, the moist equivalent of 30 kg dry soil was weighed into a large plastic bin. Compost (290 g moist compost pot^{-1}, equivalent to 1200 mg C kg $soil^{-1}$ and 70 mg N kg $soil^{-1}$) and finely powdered elemental S (2 g kg $soil^{-1}$) were added in factorial combinations to yield the non-stressed treatments C-S- (no-compost, no-S control) and C+S- (+ compost, no-S), and the stressed treatments C-S+ (no-compost, + S) and C+S+ (+ compost, + S). Amendments were mixed thoroughly by hand with the sieved soil and then packed into nursery pots (35 cm diameter, 30 cm height) to a uniform bulk density of 1.0 g cm^{-3}. Control pots without amendments (treatment C-S-) were mixed in a similar manner. Within each block, two pots were prepared for each treatment so that sufficient soil could be obtained at subsequent sampling dates without changing the hydraulic properties by over-sampling a single pot. Pots were randomized within blocks and placed on greenhouse benches. For each treatment, additional material amended at the same rates was prepared and reserved in covered buckets.

Two angled drip emitter stakes (2 L hr^{-1}, Netafim, Israel) were placed in each pot to a depth of about 7 cm. Pots were initially irrigated to saturation and allowed to drain. Subsequently pots were drip-irrigated as needed to maintain an average soil moisture of about 30 to 45% water-holding capacity. Cracking, ponding, and preferential flow leaching were not observed. The greenhouse did not receive artificial lighting or heating. Average air temperature during the trial period was 17°C. Pots were maintained under these conditions for 60 d, after which stress was partly relieved in the S+ pots by adding 60 ml of liquid lime (CalFlo, 0.64 calcium-carbonate equivalent, density= 1.77 g ml^{-1}) suspended in 3 L of water, followed by leaching with an additional 6 L of water. The S- control pots were also leached with 9 L of water. Three days later, all pots were re-leached with an additional 9 L of water. The total volume of leaching water corresponded to roughly 1.7 pore volumes.

Soil sampling

For baseline analyses, about 1 kg of soil was taken from each block prior to treatment application and immediately placed on ice and kept at 4°C until further analysis. Soil was sampled at 21 d and 42 d

after S and compost application ("Early Stress" and "Late Stress") from the top 18 cm of each pot using a 2 cm diameter soil probe. To ensure consistent moisture and aeration conditions, at each date a single sample was taken from each pot at a distance of 7.6 cm from a drip emitter and at least 7.6 cm from the pot wall. Sampling holes were backfilled with the previously amended soil and marked to avoid re-sampling. The samples from the two duplicate pots within each block were composited, and samples were put on ice and kept at 4°C until analysis. At 8 d after the final leaching, a third sampling ("Alleviation") was performed using the same method.

At each date soils were sieved to 4.75 mm on the day of sampling, and a subsample was dried at 105°C overnight to determine moisture content. At the "Baseline" sampling only, water holding capacity (WHC) was determined as the gravimetric water content of soil that had been saturated and let to freely drain for 1 hr. The soil 60% WHC was calculated using the average WHC from all five blocks and used for all subsequent incubations.

Microbial C and N cycling assays on moist soils

A set of assays was performed at each sampling date to assess microbial C and N cycling functions with and without the addition of fresh residues. Measured and calculated parameters are summarized in Appendix 2 Table 1. Within 1 day of each sampling, 6 subsamples of 6 g of moist sieved soil were weighed into separate 40-ml glass vials (vials A through F). Vial A was extracted immediately with 30 ml 0.5 M K_2SO_4. Vial B was fumigated with chloroform for 24 hours, and then similarly extracted. To vials C and D, powdered bell bean residue (*Vicia faba*, L.; 3.9% N, C:N=10.5) was added at a rate of 2.5 mg g^{-1} dry soil, corresponding to 1058 mg C kg^{-1} dry soil and 98 mg N kg^{-1} dry soil. Residue was mixed thoroughly with the soil, vials were adjusted to 60% WHC with deionized water and placed together uncovered into a 907-ml glass jar with an airtight lid fitted with a rubber septum for headspace sampling. Vials E and F were treated similarly, except that no residue was added. Headspace CO_2-C was measured after 24 h, 72 h and 7 d using an infrared gas analyzer (Qubit Systems, Canada). Jars were aerated after each sampling event. After 7 d vials C and E were immediately extracted with K_2SO_4 as described above, while vials D and F were fumigated with chloroform and then extracted. Extracts from the unfumigated vials A, C, and E were analyzed for ammonium (NH_4-N) and nitrate (NO_3-N) using the

salicylate method (Verdouw et al., 1978; Forster, 1995) and a single reagent method (Doane and Horwath, 2003), respectively. Extracts from all six vials were analyzed for total organic C (TOC) using a TOC Analyzer (Shimadzu Corporation, Japan). Microbial biomass C before the incubation (MBC_0), after incubation with residues (MBC-Res), and after incubation without residues (MBC-Soil) was calculated as the difference in TOC between the fumigated and unfumigated extracts of vials A and B, C and D, and E and F respectively, according to Horwath and Paul (1994). Initial mineral N (Mineral N_0) was calculated as the sum of NH_4-N and NO_3-N in vial A. Net N mineralization from SOM (Nmin-Soil) was calculated as the difference between mineral N in vials E and A, and the C mineralization from SOM (CO_2-Soil) as the cumulative C evolved from the jar containing unamended vials E and F. Apparent C (CO_2-Res) and net N (Nmin-Res) mineralization in response to residue addition was calculated as the difference in respired C or mineral N between vials incubated with and without residue additions, while apparent MBC growth in response to residue additions (MBC-Res) was calculated as the MBC difference between vials incubated with and without residue additions. Baseline and unamended mineralization measurements were calculated as mg C or N per kg of dry soil, while mineralization and growth responses for amended soils were calculated as a percentage of the added residue C or N. The proportion of NH_4-N in the mineral N (NH_4-N plus NO_3-N) measured in vial C after 7 d (NH_4-Res) was used to assess nitrification activity.

Chemical and biological measurements on dried soils

On the same day as the incubation setup, the remainder of the soil was air-dried at room temperature by spreading in a single-aggregate layer. Dried soils were then ground to pass through a 2-mm sieve. The EC and pH were measured in a 2:1 water:soil slurry (Thomas, 1996). Potential activities of the enzymes β-glucosidase (BG_0) and N-acetyl-β-glucosaminidase (NAG_0) were measured according to Tabatabai (1994) and Parham and Deng (2000), respectively. Microbially-available pools of C and N were assayed by extracting 6 g of soil with 30 ml of 0.01 M $CaCl_2$ (Self-Davis et al., 2000), using a filter paper with 5 to 10 µm particle retention (Fisherbrand, Q5). Subsamples of filtered dilute salt extracts were analyzed for total organic C ($DSOC_0$) and mineral N as described above. Total dissolved N in the extracts was analyzed using the persulfate digestion method (Cabrera and Beare, 1993). Organic N in the dilute salt extract ($DSON_0$) was calculated as the difference between the total dissolved and mineral N pools. As an index of Al toxicity, 10 g of soil were extracted with 20 ml 0.01 M $CaCl_2$ and filtered through paper with

a 5-10 µm particle retention (Bertsch and Bloom, 1996). Total soluble Al (Al_{tot}) was measured on extracts in 2% nitric acid solution using an inductively coupled plasma optical emission spectrometer (ICP-OES; Thermo Scientific, Waltham, MA) at a wavelength of 167.08 nm (Kerven et al., 1989). Soluble monomeric Al (Al_{mono}) was measured using the pyrocatechol violet method with a read time of 60 s, as described by Kerven et al. (1989). Organically complexed soluble Al was calculated as the difference between Al_{tot} and Al_{mono} (Mokolobote and Haynes, 2001).

Additional physical and chemical analyses were all measured on the baseline soils (Table 2.1). These included total C and N by dry combustion (Nelson and Sommers, 1996), texture by the pipet method (Gee and Bauder, 1996), bicarbonate-extractable phosphorus (Watanabe and Olsen, 1965), and extractable base cations by the ammonium acetate method (Helmke and Sparks, 1996; Suarez, 1996).

Table 2.1 Baseline soil properties. Electrical conductivity (EC) and pH were measured in 2:1 water:soil slurry. Mg, Ca, K and Na were determined in ammonium acetate extracts. s.e.= standard error (n=5).

	Avg	s.e.
Total C (g kg^{-1})	8.03	0.13
Total N (g kg^{-1})	1.11	0.07
Sand (%)	32.50	0.96
Clay (%)	26.65	2.52
NH_4-N (mg kg^{-1})	0.68	0.04
NO_3-N (mg kg^{-1})	12.2	1.28
EC (µS cm^{-1})	106	10.5
pH	7.44	0.04
Olsen P (mg kg^{-1})	13.6	0.20
Mg (mg kg^{-1})	1528	20.3
Ca (mg kg^{-1})	1818	13.2
K (mg kg^{-1})	349	5.71
Na (mg kg^{-1})	14.0	1.35

Statistical analyses

The main and interactive effects of compost and S treatments and sampling date were analyzed as a three-way ANOVA in a randomized complete block design with compost, S and date as fixed effects and block as random, using PROC GLIMMIX in SAS (SAS Corporation, Cary, NC). Differences between treatments were assessed by Tukey's honestly significant difference (HSD) test for multiple comparisons among treatments (α=0.05). To interpret interactions among fixed effects, SLICE statements were used to

partition LSMEANS and significance was assessed using Tukey adjusted p-values. The "Baseline" sampling date was not included in the statistical analysis, as the sampling unit was not the same. Date was considered a repeated measure (REPEATED), with "pot" as subject. For each parameter the covariance structure which resulted in the lowest Akaike Information Criterion was selected. To test the hypothesis that compost would reduce the effect of stress, the difference between compost treatments for the stressed soils (C-S+ and C+S+) was assessed at each date and across all three sampling dates using contrast statements. Data were log-transformed prior to analysis if necessary to meet the assumptions of normal, independently distributed residuals and equal variance. The linear relationship between MBC and either soil pH or monomeric Al in stressed soils was assessed using PROC REG in SAS. The residuals were examined visually to determine if assumptions were met.

2.3 RESULTS

Effect of sulfur addition on soil pH, salinity, and soluble Al

Sulfur addition decreased pH from a slightly alkaline value of 7.4 to 4.6 ($p<0.0001$; Fig. 2.1a), which is considered strongly acidic (Brady and Weil, 2002). Early and Late Stress dates were not significantly different for S+ soils, but stress alleviation increased pH to approximately 6 ($p<0.0001$). Additions of compost did not affect the pH for either S treatment at any date. Sulfur addition increased salinity in 2:1 suspension from a baseline of about 100 µS cm^{-1} to 1500 - 3000 µS cm^{-1} during Early and Late Stress (Fig. 2.1b). This is equivalent to roughly 4 to 8 dS m^{-1} in a saturated paste extract (Hogg and Henry, 1984). Soils in this range are considered to be slightly to moderately saline (Brady and Weil, 2002). Salinity increased between Early and Late Stress for both S+ ($p<0.0001$) and S- ($P=0.01$) treatments. After alleviation salinity in the stressed soils returned to the same level as Early Stress, while leaching the controls reduced salinity below their level at Early Stress ($p<0.0001$). Across all dates and S levels, compost treatments (C+) were slightly but not significantly more saline than non-compost treatment (C-; $p=0.10$).

Monomeric soluble Al (Al_{mono}) began to increase below pH 5.5 and increased exponentially as pH decreased further (Fig. 2.2). Concentrations of Al_{mono} tended to be higher at Late Stress than Early Stress, though the difference was not significant. Total soluble Al (Al_{tot}) generally followed the same

pattern. The organically bound soluble Al tended to increase with increasing pH, being on average 20% of Al_{tot} at pH<5.5 and 60% at pH>5.5.

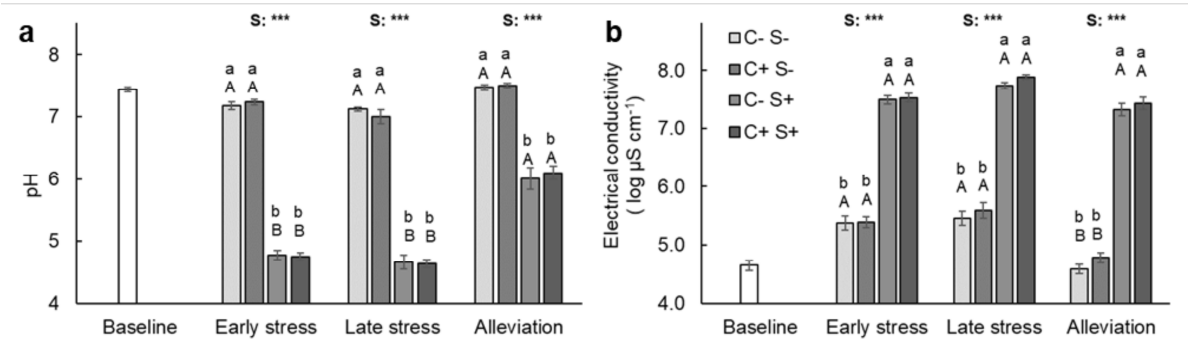

Figure 2.1. Soil a) pH and b) Electrical conductivity for soils with and without compost (C+ and C-) and sulfur (S+ and S-) additions prior to incorporation (Baseline), 21 and 42 d after incorporation (Early and Late stress), and 7 d after leaching with liquid lime (Alleviation). Different lower-case letters denote significant ($p<0.05$) differences among treatments at each sampling date, and different uppercase letters denote significant differences for a single treatment among sampling dates. Asterisks show the significance of the main effect of sulfur for each date. Error bars denote the standard error of the mean. Asterisks *** denote $p<0.001$.

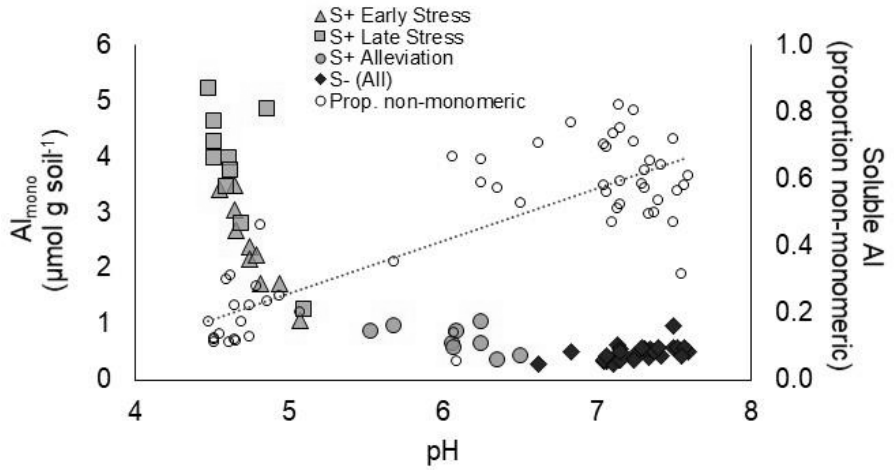

Figure 2.2. Relationship between pH in 2:1 water:soil slurry and monomeric Al (Al_{mono}) in 0.01 M $CaCl_2$ extracts as well as the proportion of the soluble Al in the non-monomeric form.

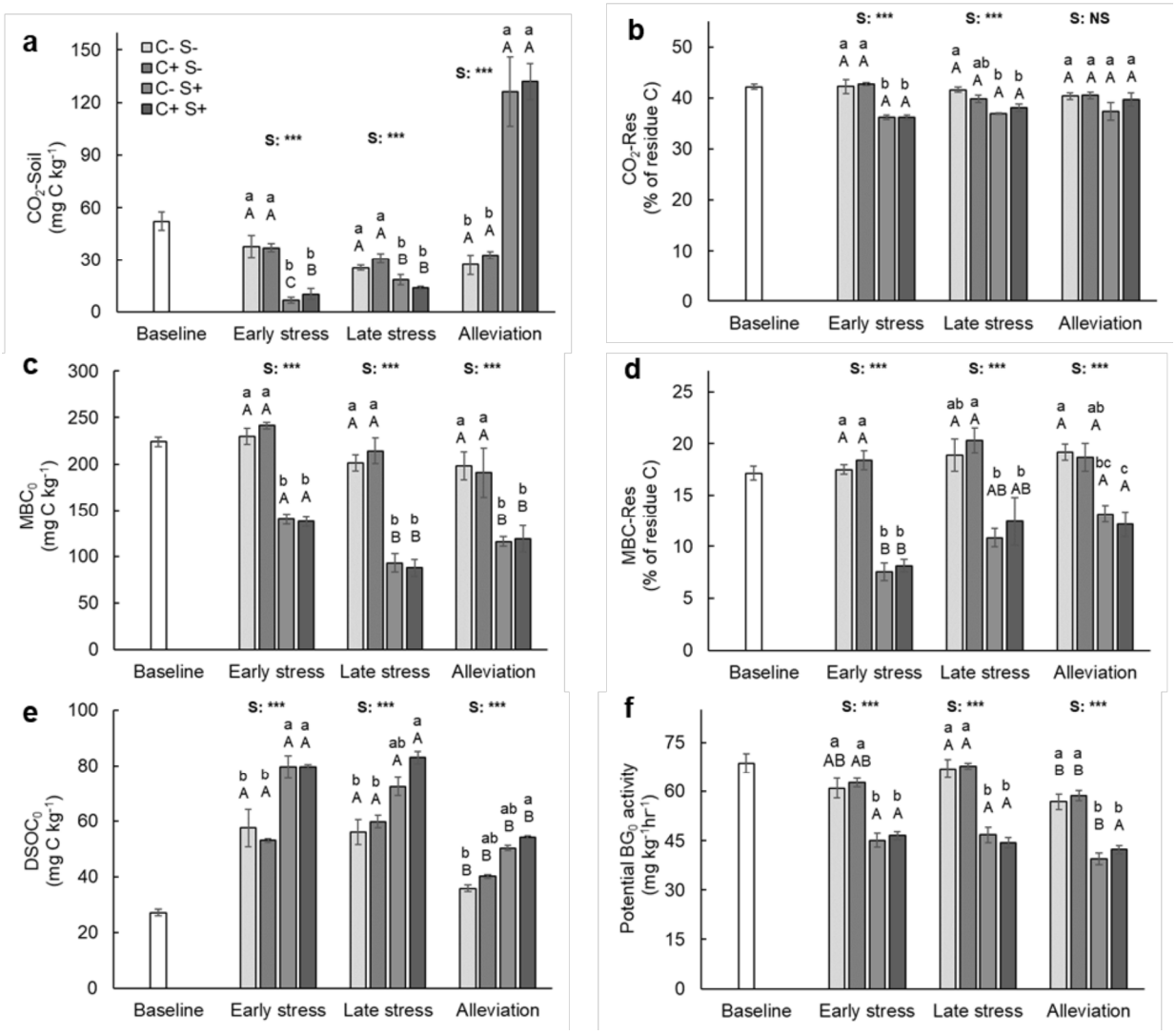

Figure 2.3. Carbon cycling and pools indicators prior to compost (C+ or C-) and sulfur (S+ or S-) incorporation (Baseline), 21 and 42 days after incorporation (Early and Late Stress), and 7 d after leaching with liquid lime (Alleviation). Asterisks *** denote Tukey-adjusted p-values of <0.001 for the main effect of S at each date. Different lower-case letters denote significant ($p<0.05$) differences among treatments at each sampling date, and different uppercase letters denote significant ($p<0.05$) differences for a single treatment among sampling dates. CO_2-Soil=CO_2-C respired during a 7-d incubation at 25°C without residue addition, and CO_2-Res=additional respiration with residue addition, compared to CO_2-Soil. MBC_0= initial Microbial Biomass Carbon. MBC-Res= MBC increase during a 7-d incubation with residues at 25°C compared to a no-residue control. $DSOC_0$= initial dilute-salt extractable organic C. BG_0=initial β-glucosidase activity.

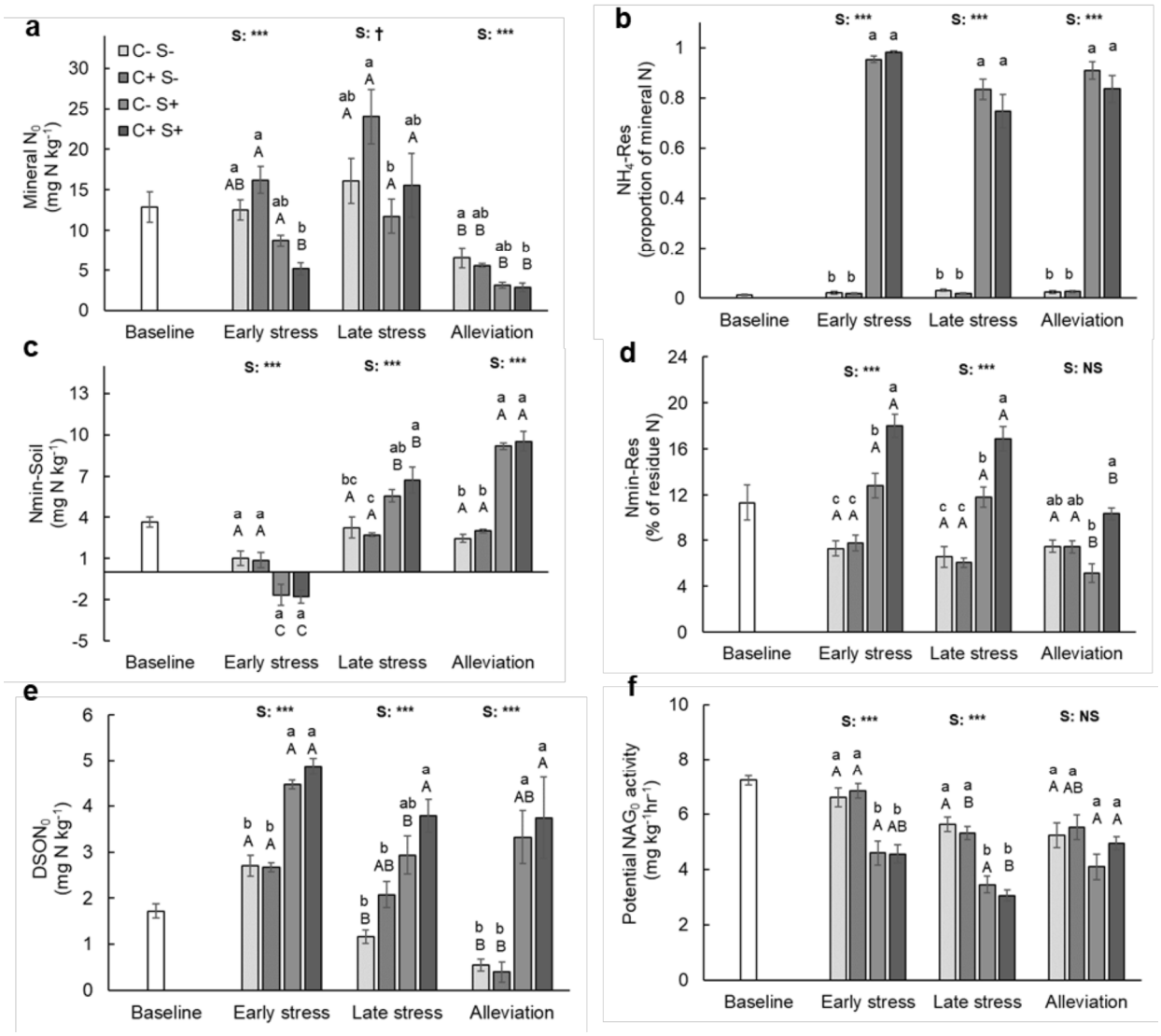

Figure 2.4. Nitrogen cycle indicators prior to compost (C+ or C-) and sulfur (S+ or S-) incorporation (Baseline), 21 and 42 days after incorporation (Early and Late Stress), and 1 week after leaching with liquid lime (Alleviation). Dagger† and asterisks*** denote Tukey-adjusted p-values of <0.1 and <0.001 for the main effect of S. Different lower-case letters denote significant ($p<0.05$) differences among treatments at each sampling date, and different uppercase letters denote significant ($p<0.05$) differences for a single treatment among sampling dates. Mineral N_0=Initial mineral N. Nmin-Soil=Net N mineralization from soils incubated without residue additions for 7 d at 25°C. Nmin-Res=additional N measured after incubation when residues were added, compared to Nmin-Soil. NH_4-Res=proportion of mineral N in soil incubated with residues for 7 d measured as NH_4-N. $DSON_0$= dilute-salt extractable organic N. NAG_0=N-acetyl-β-glucosaminidase activity.

Changes in non-stressed controls

For both S- control treatments, most parameters remained constant over the course of the incubation, with the exception of $DSOC_0$, BG_0, and Mineral N_0, all of which were lower after leaching (Figs. 2.3 and 2.4).

Early response to S-induced stress (21 days after treatment)

All C parameters were significantly reduced by S at Early Stress (Fig. 2.3a-f), with the exception of $DSOC_0$, which was on average 44% higher in the S+ treatments than the S- treatments (Fig. 2.3e). The most affected parameter was CO_2-Soil, which was reduced by 75% in S+ compared with S- treatments (Fig. 2.3a). The least affected parameter was CO_2-Res, which was only reduced by 15% (Fig. 2.3b). As a result, the stimulatory effect of residues on respiration (ratio of CO_2-Res to CO_2-Soil) at Early Stress was more than five times higher in the S+ treatments than in the S- treatments ($p<0.0001$, data not shown). The MBC_0 was reduced by 41% and the MBC-Res by 56% in the S+ compared to the S- treatments, while potential BG_0 activity was reduced by 25% (Fig. 2.3c,d,f).

Like the C parameters, all measured N pools and processes were strongly affected by S addition at Early Stress, (Table 2.2; Fig. 2.4a-f). More than 90% of the mineral N (NO_3-N + NH_4-N) in the residue-added soils was in the form of NH_4^+ in the S+ treatments, compared with 2% in the S- treatments, suggesting inhibition of nitrification (Fig. 2.4b). Mineral N_0 in the S+ treatments was less than half of that in the S- treatments (Fig. 2.4a), potential NAG_0 activity was reduced by about 30% (Fig. 2.4f), and Nmin-Soil was negative (Fig. 2.4c). In contrast, Nmin-Res was more than doubled on average in the S+ compared to S- treatments (Fig. 2.4d), although there was a significant interaction with compost (Table 2.3; discussed below). The $DSON_0$ was on average 75% higher in the S+ treatments than the S- treatments (Fig. 2.4e).

Longer term response to S-induced stress (42 d after treatment)

Across the two compost treatments, all C indicators remained significantly lower in the S+ than the S- treatments after 42 days of stress, except $DSOC_0$, which remained higher (Fig. 3a-f). However, CO_2-Soil significantly increased in the S+ treatments, doubling between Early and Late Stress (Fig. 2.3a; $p<0.0001$). The increase was only significant in the absence of compost (C-S+ treatment). Mean MBC-

Res in the S+ treatments increased by 48% ($p=0.0008$), although the difference was not significant for the individual treatments (Fig. 2.4d). Conversely, the MBC_0 declined by about 35% between Early and Late Stress in the S+ treatments (Fig. 2.4c; $p<0.0001$). All other parameters remained unchanged or had non-significant increases between the two dates. Across Early and Late Stress in the S+ treatments, MBC_0 had a strong (adjusted $R^2=0.47$, $p=0.0007$) and weak (adjusted $R^2=0.17$, $p=0.04$) negative correlations with Al_{mono} and pH, respectively (data not shown).

The trend towards increased mineralization during Late Stress was more pronounced for N than C (Fig. 4c,d). Between Early Stress and Late Stress the Nmin-Soil sharply increased in the S+ treatments, becoming on average approximately double that of the S- treatments (Fig. 2.4c). Mineral N_0, though still lower in S+ than S- treatments, also significantly increased in S+ treatments from Early to Late Stress (Fig. 2.4a; $p=0.0094$). The difference was only significant with addition of compost. The Nmin-Res continued to be significantly elevated in the S+ treatments, though it did not increase from Early to late Stress levels (Fig. 2.4d). The $DSON_0$ decreased significantly between Early and Late Stress (Fig. 2.4e; $p=0.0004$), but remained significantly higher in the S+ than S- treatments ($p=0.0001$). Potential NAG_0 activity also decreased from Early to Late Stress (Fig. 2.4f; $p<0.0001$) and was significantly lower in S+ than S- treatments ($p=0.0003$).

Table 2.2 Fixed effects of Compost, Sulfur, Date, and their interactions (Tukey-adjusted p-values). $DSOC_0$= Initial dilute-salt extractable organic C. BG_0=initial β-glucosidase activity. MBC_0= initial microbial biomass C. MBC-Res= MBC increase during an incubation with residues compared to a no-residue control. CO_2-Soil and CO_2-Res= CO_2-C respired during an incubation without and with residue additions, respectively. $DSON_0$= initial dilute-salt extractable organic N. NAG_0=initial N-acetyl-β-glucosaminidase activity. Mineral N_0=Initial mineral N. Nmin-Soil and Nmin-Res=Net N mineralization from soils incubated without and with residue additions. NH_4-Res=proportion of mineral N in soil incubated with residues for measured as NH_4-N. Samples were incubated for 7 d at 25°C.

	Carbon cycle indicators						Nitrogen cycle indicators					
Effect	$DSOC_0$	BG_0	MBC_0	MBC-Res	CO_2-Soil	CO_2-Res	$DSON_0$	NAG_0	Mineral N_0	Nmin-Soil	Nmin-Res	NH_4-Res
Compost	0.036	0.242	0.353	0.520	0.637	0.214	0.021	0.615	0.749	0.654	<.0001	0.171
Sulfur	<.0001	<.0001	<.0001	<.0001	0.020	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Compost*Sulfur	0.491	0.889	0.212	0.990	0.845	0.114	0.676	0.870	0.030	0.423	<.0001	0.496
Date	<.0001	<.0001	<.0001	<.0001	<.0001	0.940	<.0001	<.0001	<.0001	<.0001	<.0001	0.284
Compost*Date	0.080	0.296	0.996	0.296	0.788	0.468	0.175	0.030	0.113	0.797	0.906	0.132
Sulfur*Date	0.368	0.199	0.144	0.001	<.0001	0.006	0.259	0.001	0.337	<.0001	<.0001	0.038
Compost*Sulfur*Date	0.716	0.379	0.956	0.898	0.236	0.199	0.773	0.531	0.141	0.483	0.943	0.271

Table 2.3 Comparison of compost treatments for the sulfur-stressed soils at Early Stress, Late Stress, and Alleviation, and across all three dates (p-values for estimate statements, using Tukey's adjustments for multiple comparisons). $DSOC_0$= Initial dilute-salt extractable organic C. BG_0=Initial β-glucosidase activity. MBC0= initial microbial biomass C. MBC-Res= MBC increase during an incubation with residues compared to a no-residue control. CO_2-Soil and CO_2-Res= CO_2-C respired during an incubation without and with residue additions, respectively. $DSON_0$= initial dilute-salt extractable organic N. NAG_0=initial N-acetyl-β-glucosaminidase activity. Mineral N_0=Initial mineral N. Nmin-Soil and Nmin-Res=Net N mineralization from soils incubated without and with residue additions. NH_4-Res=proportion of mineral N in soil incubated with residues measured as NH_4-N. Samples were incubated for 7 d at 25°C.

	Carbon cycle indicators						Nitrogen cycle indicators					
Date	$DSOC_0$	BG_0	MBC_0	MBC-Res	CO_2-Soil	CO_2-Res	$DSON_0$	NAG_0	Mineral N_0	Nmin-Soil	Nmin-Res	NH_4-Res
Early Stress	0.944	0.488	0.765	0.424	0.488	0.901	0.050	0.940	0.014	0.884	<.0001	0.915
Late Stress	0.059	0.317	0.717	0.413	0.104	0.336	0.072	0.354	0.290	0.169	<.0001	0.402
Alleviation	0.287	0.030	0.890	0.419	0.609	0.062	0.609	0.050	0.631	0.655	<.0001	0.599
Overall	0.099	0.440	0.831	0.648	0.849	0.047	0.055	0.639	0.181	0.393	<.0001	0.529

Stress effects seven days after alleviation by leaching with liquid lime

Microbial biomass, respiration, and the $DSOC_0$ pools had different responses to leaching with lime (Fig. 2.3a-f). Despite the increase in pH and decrease in Al_{mono}, MBC_0 and BG_0 showed no increase at Alleviation for S+ treatments (Fig. 2.3c,f). At Alleviation, these parameters were still on average 40% and 30% lower in S+ than S- treatments respectively, and the difference between S+ and S- did not vary with date (Table 2.2). MBC-Res after Alleviation was still on average 44% lower in the S+ than S- treatments, and was not significantly higher than at Late Stress (Fig. 2.3d). However, unlike MBC_0 and BG_0, MBC-Res gradually increased over the course of the experiment, and was 60% higher after Alleviation than at Early Stress ($p<0.0001$). In contrast, CO_2-Soil for S+ treatments was dramatically higher after Alleviation compared to both S- soils and S+ soils during stress (Fig. 2.3a). CO_2-Res differed neither between S+ and S- soils at Alleviation, nor between Late Stress and Alleviation for any of the treatments (Fig. 2.3b). $DSOC_0$ was significantly higher in S+ than S- treatments during both stress dates and after alleviation. After leaching the $DSOC_0$ was significantly ($p<0.0001$) lower in all soils regardless of S addition, such that the differences between S+ and S- treatments were consistent across all sampling dates (Fig. 2.3e; Table 2.2).

Between Late Stress and Alleviation, the N cycling parameters in the S+ treatments either increased (Nmin-Soil, NAG_0; Fig. 2.4c,f), decreased (Mineral N_0, Nmin-Res; Fig. 2.4a,d), or remained stable (NH_4-Res, $DSON_0$; Fig. 2.4b,e). Interestingly, in S+ treatments Nmin-Soil and Nmin-Res showed reversed trends across the experiment, with Nmin-Soil lowest at Early Stress and steadily increasing until Alleviation, and Nmin-Res high during stress but significantly lower after liming. By Alleviation, Nmin-Soil in S+ was on average more than three times higher than in S- (Fig. 2.4c), while Nmin-Res did not differ (Fig. 2.4d).

Effect of compost on stress response and recovery

Compost had little to no effect on most microbial parameters measured. Averaged across all S treatments and dates, only $DSOC_0$ and $DSON_0$ were significantly increased by the compost treatment (i.e., significant main effect with no interactions; Table 2.2). Compost had a large impact on Nmin-Res ($p<0.0001$) which changed with S addition (Table 2.2). The compost treatment strongly increased Nmin-

Res in the S+ soil at all dates (Table 2.3; Fig. 2.4), with C+S+ being 40%, 42%, and 100% higher than C-S+ at Early Stress, Late Stress, and Alleviation, respectively ($p<0.0001$; Fig. 2.4d; Table 2.3). No corresponding difference was observed in the S- treatments. All other compost effects in the stressed soils were slight and temporary (Table 2.3). These include CO_2-Res, which was significantly higher in the C+S+ than C-S+ treatment averaged over all the dates but was not significant at any individual date (Fig. 2.3b; Table 2.3), and Mineral N_0, which was significantly lower in C+S+ than C-S+ at Early Stress (Fig. 2.4a; Table 2.3).

2.4 DISCUSSION

Low soil pH likely caused severe stress to the soil microbial community. The minimum pH reached in the acidified soil (4.6) was near the threshold of 4.5 below which all bacterial and fungal growth were observed to be heavily impacted at a long-term agricultural pH gradient, even when stimulated with substrate additions (Aciego Pietri and Brookes, 2009; Rousk et al., 2010b). The authors attributed this inhibition to the high levels of available Al observed at this pH. In our study, soluble Al began to increase exponentially below pH 5.5. While the Al_{mono} concentrations were low, the significant negative correlation with microbial biomass during Early and Late Stress suggests Al toxicity may have been a stressor (Jones et al., 2019). Excess protons themselves pose physiological challenges to microbes (Lund et al., 2014), and soil bacterial communities have been observed to shift transcriptional priorities from growth to repair and maintenance at pH<6.2 (Malik et al., 2018). While inter-study salinity comparisons are difficult due to strong effects of moisture, texture, and the method used to measure electrical conductivity, our salinity values are at the lower end of those observed to impact microbial growth and activity (Pathak and Rao, 1998; Rath et al., 2019a; Yan and Marschner, 2012). Therefore, soil pH was likely a stronger stress than salinity in our study.

Our goals were to monitor how a strong, short-term stress caused by the addition of elemental S would affect microbial community function, the extent to which function would recover after partial correction, and how those dynamics would be moderated by compost addition. We observed that patterns of initial response, adaptation, and recovery differed strongly between different C and N cycling functions. Four clear responses to S-induced stress were observed: an almost complete inhibition of nitrifier activity,

a long-lasting increase in $DSOC_0$ and $DSON_0$ pools, an apparent decoupling of C and N mineralization responses, and a pronounced, consistent increase in net N mineralization from a labile residue addition in the compost-amended stressed soils.

Inhibition of nitrifying activity under stress

The strongest functional change due to S addition was an almost complete absence of nitrification from added residues. This is in line with several long-term studies which consistently observed lower nitrification in both acid and saline soils (e.g. Aciego Pietri and Brooks, 2008a; Cheng et al., 2013; Kemmitt et al., 2006; Pathak and Rao, 1993). At low pHs, the direct mechanism is thought to be a lack of NH_3 substrate for ammonia monooxygenase, the main enzyme responsible for NH_3 oxidation (Li et al., 2018). Nitrification has also been observed to be limited by free Al (Kraal et al., 2009). While nitrification can occur efficiently in some acid soils, this appears to be carried out by a compositionally different community than that in neutral soils, rather than a similar community with stress-induced adaptations (Li et al., 2018). The fact that liming did not appear to increase nitrification capacity suggests that the inhibition at this point was due more to the loss or slow recovery of nitrifier community members than the lack of substrate or acid cation toxicity. Nitrification is a multistep process, carried out by different groups of rather specialized organisms, and successful nitrification depends on their coupled function (Norton and Stark, 2011; Li et al., 2018). Our results reinforce the conclusions of Chaer et al. (2009) that narrow niche functions are more easily lost from a stressed community than broadly distributed functions such as mineralization and may therefore be more sensitive indicators of soil ecological stability.

Increase in extractable organic matter under stress

When naturally acid soils are limed, organic matter in the soil solution usually increases as organic matter functional groups are deprotonated and become less attracted to negatively charged mineral surfaces (Andersson et al., 1994; Curtin et al., 1998; Evans et al., 2012). The high $DSOC_0$ and $DSON_0$ in the S+ treatments show that the reverse does not necessarily hold when neutral soils are acidified. Similarly, Kemmitt et al. (2006) observed that dissolved organic C and N were negatively correlated with pH, microbial biomass and respiration and closely positively correlated with exchangeable Al across two long-term pH gradients. A plausible explanation is that respiration and the ability to

metabolize soluble organic matter were limited by acid cation toxicity (Hue, 2011). In addition, microbial strategies for surviving low pH include the release of low-molecular weight organic acids and extracellular polymeric substances to complex with and sequester free Al (Auger et al., 2013; Draghi et al., 2016). Although our work was not designed to address this question it is possible that these substances directly contributed to the elevated $DSOC_0$ and $DSON_0$, as well as the microbial necromass. While complexation with Al may also reduce organic matter's susceptibility to microbial attack (Álvarez et al., 2012; Haynes and Mokolobote, 2001; Hue, 2011; Scheel et al. 2007), the very low proportion of complexed soluble Al at low pH suggests this mechanism was not an important contributor to the higher concentrations of $DSOC_0$ and $DSON_0$ in the acidified soil. Finally, we observed that potential enzyme activities were reduced in the S+ treatments compared to the S- treatments, but actual activity may have been higher due to the low pH optima of both BG_0 and NAG_0 (Parham and Deng, 2000; Turner, 2010).

Carbon and nitrogen cycle responses: acute toxicity during Early Stress

As predicted in our first hypothesis, stress strongly reduced microbial biomass and activity. The more marked decline in respiration than biomass at Early Stress is interesting, as a high metabolic quotient is often considered to be an indicator of soil stress (i.e. Anderson and Domsch, 1993; Rietz and Haynes, 2003; Yuan et al., 2007). However, the joint inhibition of respiration and growth may be a sign of a poorly adapted community dealing with specific toxicity, in which part of the community becomes dormant or dies, rather than a generally stressful one in which the microorganisms must expend more energy to survive (Rath et al., 2016). The smaller decline in MBC_0 than CO_2-Soil may also be partly explained by the fact that the chloroform fumigation extraction (CFE) method of assessing microbial biomass C measures cytoplasmic C released after the dissolution of cell membranes (Horwath and Paul, 1996), and thus would include any accumulation of stress-induced molecules in the cytoplasm (i.e. Draghi et al., 2016; Gale, 1946; Kakumanu and Williams, 2014; Lund et al., 2014; Schimel et al., 1989).

Intriguingly, the negative values for Nmin-Soil in the S+ treatments and low Mineral N_0 at Early Stress suggest the small microbial community was immobilizing N, since significant denitrification or volatilization are unlikely in recently acidified soils with low organic matter and NO_3^- concentrations under aerobic conditions (Bremner and Shaw, 2002; Šimek et al., 2002). Our finding that microorganisms

depleted the mineral N pool while the $DSON_0$ pool increased suggests preferential uptake of mineral N. An increased need for N under acute stress would be consistent with pure culture studies, which have observed that as bacteria shift from growth to survival mode under acid stress, there is marked increase in the synthesis of proteins and proteases (Draghi et al., 2016). New enzymes are required for repair, maintenance, and to catalyze proton-sequestering reactions, new membrane pumps must be built, and damaged proteins and DNA must be fixed or broken down and recycled (Dilworth and Glenn, 2007; Draghi et al., 2016; Gale, 1946; Lund et al., 2014).

Legume residue additions, however, dramatically increased both C and N mineralization responses in the S+ treatments at Early Stress, with Nmin-Res from S+ treatments exceeding that from S- treatments. This suggests that the residues somewhat relieved the acute toxicity which had been limiting respiration. At the same time, MBC-Res remained relatively low, resulting in the reduced substrate use efficiency, which often characterizes stressed communities (Anderson and Domsch, 1993). Fresh residues (particularly legumes) increase organic acid concentrations in the soil solution, complexing with Al and reducing its activity (Mokolobote and Haynes, 2002; Hue, 2011, Kretzschmar et al., 1991; Xiao et al., 2014). Addition of legume residues also temporarily consumes protons, through decarboxylation and ammonification of soluble organic acid anions (Dilworth and Glenn, 2007; Xiao et al., 2014; Xu et al., 2006). As these reactions produce CO_2 and NH_4^+, respectively (Gale, 1946; Haynes and Mokolobote, 2001; Xu et al., 2006), this detoxification response likely contributed to the observed C and N mineralization pulse. In addition, residue additions to very acid soils have been observed to stimulate fungal but not bacterial growth (Rousk et al., 2010b), suggesting that the residue additions may have fueled a community shift to one with a higher C to N ratio (Strickland and Rousk, 2010). For example, Silva-Sánchez et al. (2019) observed rapid fungal but not bacterial growth within four days of a residue addition to acid soils. Combined with the reduced metabolic efficiency and potential for acid tolerance responses which generate NH_4^+, a higher fungal growth response could help explain why Nmin-Res in the S+ treatments was on average more than double that in the S- treatments.

Carbon and nitrogen cycle responses: community adaptation during Late Stress

The diminished MBC_0 but generally increased ability to mineralize C and N and to respond to residue additions observed at Late Stress suggests a smaller but more stress-adapted community (Cruz-Paredes et al., 2017; Rath et al., 2019a). Under conditions of abundant substrate availability, significant shifts in community tolerance to acidity have been observed within 36 d (Cruz-Paredes et al., 2017) and within 15 d for salinity (Rath et al., 2019a). The apparent tradeoff between respiration and biomass is consistent with the idea that communities adapted to stressful environments are less energetically efficient (i.e. Malik et al., 2018; 2019), as they must maintain the specialized membrane structures, metabolites, and enzymes which allow them to survive and grow under adverse conditions (Draghi et al., 2016; Malik et al., 2018; Ramin and Allison, 2019; Schimel et al., 2007). The shift from N immobilization during Early Stress to net N mineralization from SOM, which exceeded that of the S- treatments during Late Stress, is consistent with the gradual shift to a less efficient community with a higher C to N ratio (Silva-Sánchez et al., 2019). A metabolically inefficient microbial community that mineralizes a relatively large amount of new residue C or N per unit biomass growth suggests that, in these stressed soils, less input of C or N may be retained as SOM.

Carbon and nitrogen cycle responses: limited recovery after stress alleviation

The most obvious effect of liming was a very large CO_2 pulse from the unamended soil, far exceeding the $DSOC_0$ pool. It is likely that at least part of this was abiotic, issuing from the decomposition of carbonic acid from the liming reaction to CO_2 (Bertrand et al., 2007). While it is not possible to separate biotically and abiotically generated CO_2 in this experiment, the fact that additional respiration due to residues (CO_2-Res) was remarkably similar before and after leaching suggests that liming did not increase the capacity for microbial respiration when adequate substrate was present. Contrary to our hypothesis, MBC_0 and potential BG_0 activity did not increase at Alleviation compared with Late Stress. However, the tendency towards higher MBC-Res with equivalent CO_2-Res and the significant decrease in Nmin-Res all suggest that the community that grew in response to residue additions after liming was more efficient and likely had a lower C to N ratio than that which responded at Late Stress. Interestingly, whereas Nmin-Res significantly decreased after liming in the S+ treatments, Nmin-Soil continued to

increase. This is consistent with the idea that community shifts happen more quickly in the presence of an available substrate (Rath et al., 2019a; Silva-Sánchez et al., 2019).

The fact that Nmin-Res was highest during stress and declined after liming reinforces the idea that a relatively high ability to mineralize N from an added substrate may in fact be a symptom of an inefficient, stress-adapted community. Our results are in line with several studies which found that net N mineralization was not inhibited by salinity and acidity to the same extent as C mineralization and nitrification (Aciego Pietri and Brookes, 2008a and 2008b; Cheng et al., 2013; Kemmitt et al., 2006; Laura, 1976; Pathak and Rao, 1998). Indeed, net N mineralization has sometimes been observed to be highest in the most acid soils within an experimental gradient (Aciego Pietri and Brookes, 2008a; Xiao et al., 2013). Similarly, an increase in net N mineralization from vetch residues has been measured in response to Al additions (Kraal et al., 2009). Our findings also complement two recent studies (Soares and Rousk, 2019; Silva-Sánchez et al., 2019) which concluded that when communities are dominated by fungi due to acid stress, they tend to use substrate less efficiently.

Effect of compost

Contrary to our fourth hypothesis, compost addition made no difference to C cycle functional response to or recovery from stress, despite slightly increasing the $DSOC_0$ and $DSON_0$ pools across all sampling dates. This may be because compost is generally a stable, microbially processed product, rich in condensed, high molecular weight compounds, phenols and lignin and depleted in energetic compounds such as sugars (Lerch et al., 2019; Said-Pullicino et al., 2007). Therefore, the C in compost is unlikely to be an easily available energy source. While humic substances extracted from composts have been observed to reduce free Al (Winarso et al., 2018) and consume protons in acid soils (Mokolobote and Haynes, 2002; Naramabuye and Haynes, 2006), our green waste compost did not have any discernible effect during the time scale of this experiment. It is possible that short-term responses to bioavailable fractions may have occurred prior to our first measurement 21 days after application.

Surprisingly, the only effect of compost was to strongly increase Nmin-Res, which it did consistently across all three sampling dates. It is especially interesting, given the magnitude of the effect, that corresponding differences were mostly absent in the C cycle indicators, or in the Nmin-Soil. A

possible explanation is that the compost introduced or promoted organisms with a higher C to N ratio, such that when sufficient substrate became available or toxicity constraints were removed, the growing community in the C+ treatments had a comparatively lower N requirement than that in the C- treatment. Even a strong community shift would not necessarily be evident in respiration measurements due to functional redundancy. For example, at the Hoosfield Acid Strip, fungal growth rates increased 30-fold as pH declined from 8.3 to 4.5, while respiration changed by less than one third (Rousk et al., 2009).

2.5 CONCLUSIONS

We found that a sudden chemical stress reduced microbial biomass and respiration and completely inhibited nitrification, but increased N mineralization dilute salt-extractable organic C and N pools. Our results suggest that, like more long-term stresses observed in natural systems, sudden chemically induced stress may reduce microbial anabolic efficiency, potentially decreasing the proportion of input C and N retained as organic matter. The results have implications for our understanding of how biological processes change in stressed agricultural soils. To our knowledge, our results are the first to explicitly link the decreased microbial efficiency often associated with stress to an increase in N mineralization. Given the consistency and magnitude of the increase, it has direct implications for C and N retention in agricultural soils warrants mechanistic elucidation. Surprisingly, amendment with compost had little effect on C pools and processes, but strongly increased N mineralization from labile residue additions in the stressed soils. Stress increased catabolic activity (C and N mineralization) relative to anabolic activity (microbial biomass growth) both during the stress and after its alleviation. Rather than moderating this effect, in the short term green waste compost appears to have exacerbated it by increasing the amount of N mineralized from labile residues.

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Chapter 3: Sensitivity and Variability of Soil Health Indicators in a Drip Irrigated Mediterranean Annual Cropping System.[3]

ABSTRACT

To choose or incentivize practices that build healthy soils, growers and policymakers need indicators that can reliably show whether improvements in soil health have occurred. A useful indicator for monitoring soil health over time must be sensitive enough to respond quickly to management changes, without being overly influenced by variations in sampling time or location, previous crop, or normal year-to-year differences in weather or operations timing. Our goal in this study was to assess the sensitivity and variability of a suite of soil health indicators at the Russell Ranch Century Experiment in California. These plots have been either conventionally or organically farmed in a corn-processing tomato rotation for 25 years and were expected to have developed stable differences between management systems. We took samples in both crop phases prior to planting and midseason for two consecutive years. At each sampling date we took three adjacent subsamples per plot to assess intrinsic variability for each indicator. Management was the strongest factor differentiating most indicators, particularly those relating to soil biology and carbon accumulation. While differences between management systems were generally consistent at all sampling points, absolute values varied considerably across dates and years. The crop phase of the rotation had little effect on indicators. Accounting for soil texture increased sensitivity to management for aggregate stability and most of the organic carbon (C) indicators but not the biological or most of the chemical indicators. Variables which were most sensitive to management also tended to have higher in-plot variability. Our results suggest that indicators relating to organic pools and biological processes most strongly differentiated the two systems, and underline the importance of using consistent sampling dates. They also suggest that a minimum indicator dataset which includes both relatively stable and dynamic indicators may be the most reliable to interpret.

[3] A version of this chapter has been submitted for publication under the title of "Sensitivity and variability of soil health indicators in a Mediterranean cropping system" to the Soil Science Society of America Journal. As of the date of this writing, it is under review.

3.1. INTRODUCTION

Soil health, broadly defined as “the continued capacity of a soil to function as a vital living ecosystem” (Norris et al., 2020), has been promoted as the key to producing high yields of nutritious crops while protecting the environment and fighting climate change. Accordingly, practices geared towards building soil health are increasingly being experimented with by farmers and incentivized by government and private entities. Therefore, how best to assess whether soil health is actually improving is a topic of great practical import and much recent debate (i.e. Karlen et al., 2019; Norris et al., 2020; Roper et al., 2019; Stewart et al., 2018, Wander et al., 2019).

Central to this debate are the concepts of indicator sensitivity, variability, and generalizability. A useful indicator must be able to detect improvements in some property of interest within a reasonable timeframe, while not being so affected by other factors that it is difficult to interpret or cannot be compared with other systems or over time (Hargreaves et al., 2019). For example, soil organic matter is an important component of soil health. But because it may take several years for a change to be detectable it could have limited usefulness for a farmer who wished to evaluate different management practices for increasing soil health (Hurisso et al., 2018). Conversely, plant-available nitrogen (N) is necessary for crop growth. But it changes so quickly, and is so strongly affected by a variety of climactic and edaphic factors, that it is also not very useful for assessing whether soil health is improving over time in response to management (Wander et al., 2019). Between these two extremes lie a host of potential soil health indicators that have been found to be more sensitive or less variable measures to monitor factors such as carbon (C) storage, fertility or structural stability (Hurisso et al., 2018). However, an emerging body of work suggests that the "best" metrics, as well as the appropriate sampling protocols and the thresholds used to interpret them, are likely to be affected by cropping systems, climates, and edaphic properties like texture (i.e. Caudle et al., 2020; Chahal and van Eerd, 2019; Hurisso et al., 2016; Roper et al., 2017; Wade et al., 2016; Zuber et al., 2020). Thus, as progress is being made towards developing standardized soil health assessment protocols there is a great need for regional studies which explore indicator sensitivity and variability in locally important management systems and soil types (Zuber et al., 2020). This is particularly true in California, whose Mediterranean climate and irrigated high-value crops differ

from the rainfed grain and pasture systems which are the subject of most soil health assessment research.

Our goal in this study was to assess the sensitivity and variability of a wide range of soil health indicators in the Russell Ranch Century Experiment in the Central Valley of California, a long-term experiment in which plots in a corn (*Zea mays* L.) and processing tomato (*Solanum lycopersicum* L.) rotation have been under conventional (CONV) or organic (ORG) management since 1993. Long-term research trials provide stable, controlled systems in which to demonstrate the benefit of a management practice and to help select and interpret appropriate soil health indicators (Culman et al., 2013; Diederich et al., 2019; Hurisso et al., 2016; Morrow et al., 2016). The uniform conditions allow for the systematic examination of indicators' relative susceptibility to other factors like weather. By sampling both systems in both corn and tomato phases of the rotation prior to planting and during crop growth across two years, we aim to document how soil health indicators vary with crop phase, sampling timing and year, and how those differences compare in magnitude to those caused by management. In addition, the relatively wide textural gradient which occurs across the experimental site allows us to assess how indicator sensitivity and variation is affected by soil texture.

We hypothesize that 1) Long-term organic management will result in strong, significant differences in all indicators, especially those relating to soil organic C (SOC) storage and biological function, and that 2) indicators which partly measure recent inputs will be more affected by other sources of variability (crop phase, sampling time and year) than indicators which reflect more processed fractions, such that 3) indicators with the greatest sensitivity will also have the greatest variability, and that 4) adding a textural covariate will improve the sensitivity of most indicators to management.

3.2. METHODS

Site description

The experiment was conducted in plots with a corn-tomato rotation at the Century Experiment at the Russell Ranch Sustainable Agriculture Facility in northern California (38°32'24"N, 121°52'12"W). In this experiment, a conventional management system utilizing synthetic fertilizer, pesticides, and winter fallow (CONV) is contrasted with a certified organic system with yearly application of composted poultry

manure and a winter legume cover cop (ORG). Detailed site and management information can be found in Tautges et al. (2019) and Schmidt et al. (2018). Briefly, the experiment was laid out in 1993 as a randomized complete design with three blocks. Two blocks are placed on Rincon silty clay loam soil (fine, smectitic, thermic Mollic Haploxeralfs) and the third on Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Each crop phase (corn or tomato) of each management system is represented in each block in each year on square 0.4-ha replicate plots, each consisting of 48 152.4 cm wide beds. In 2015 a single subsurface drip line was installed at a depth of 25 cm down the center of each bed. Prior to 2015 all plots were furrow irrigated. Tillage operations do not exceed 25 cm in depth.

Management during the 2018 and 2019 growing seasons

Timing of management operations for the 2018 and 2019 growing seasons is summarized in Table 3.1. In the ORG plots, a cover crop, consisting of hairy vetch (*Vicia villosa* Roth), faba bean (*Vicia faba* L.) and cereal oat (*Avena sativa* L.) was seeded in November, terminated by mowing in late February, and incorporated by disking. For the 2018 crops, composted poultry manure was broadcast in late April at the rate of 4 t ha^{-1} and incorporated. For the 2019 crop, composted poultry manure at the same rate was broadcast over the top of the corn and tomato residues in fall 2018, which were then chopped and disked to incorporate. In both years, beds were rolled to prepare the seedbeds in late April. In the ORG and CONV systems, tomatoes were transplanted down the center of the bed in early May. Corn was seeded in double rows in late April, early May in the CONV system and in late May 2018 and early June 2019 in the ORG system. The later seeding date for ORG corn was chosen because insect pressures resulted in a poor stand in 2018, requiring replanting. In the CONV system, both corn and tomato received a starter application of 56 kg N ha^{-1} as 8-24-6. No other P or K was added. In addition, urea ammonium nitrate (32%) was water-run through the drip lines several times during the growing season. In both systems, tomatoes were mechanically harvested in late August and corn in late October.

Total winter (October through March) rainfall prior to the 2018 growing season was 240 mm and prior to the 2019 growing season was 650 mm (California Irrigation Management Information System). Average air temperatures during both growing seasons (April through September) was 20°C (Fig. 3.1).

Table 3.1 Summary of crop management operations and soil and plant sampling in 2018 and 2019.

Management dates

Operation	2018	2019
Bed disking and listing	Oct (2017)	Oct (2018)
Cover crop seeding	Nov (2017)	Nov (2018)
Cover crop mow/disk/list	Feb 23	Feb 23
Compost spreading	Apr 20	Oct 24 (2018)
Compost incorporation (2018 only), cultivation, bed rolling	Apr 21 - 26	Apr 23-25
CONV corn seeding, starter NPK	Apr 21	May 04
CONV tomato starter NPK	Apr 26	Apr 25
Tomato transplanting	May 01	Apr 29
ORG corn seeding	May 25	Jun 03
Tomato harvest	Aug 30	Sep 04
Corn harvest (all)	Oct 05	Oct 11-14

Soil and plant sampling dates

Operation	2018	2019
Preplant sampling (all)	Apr 04	Apr 12
Tomato midseason soil and plant sampling	Jun 13	Jun 13
CONV corn midseason soil and plant sampling	Jun 27	Jul 05
ORG Corn midseason soil and plant sampling	Jul 20	Jul 30
Tomato bulk density sampling	Aug 14	Jul 30
Corn bulk density sampling	Oct 05	Jul 30
Tomato fruit hand-harvest	Aug 14	Aug 23
Corn grain hand-harvest	Oct 05	Oct 03

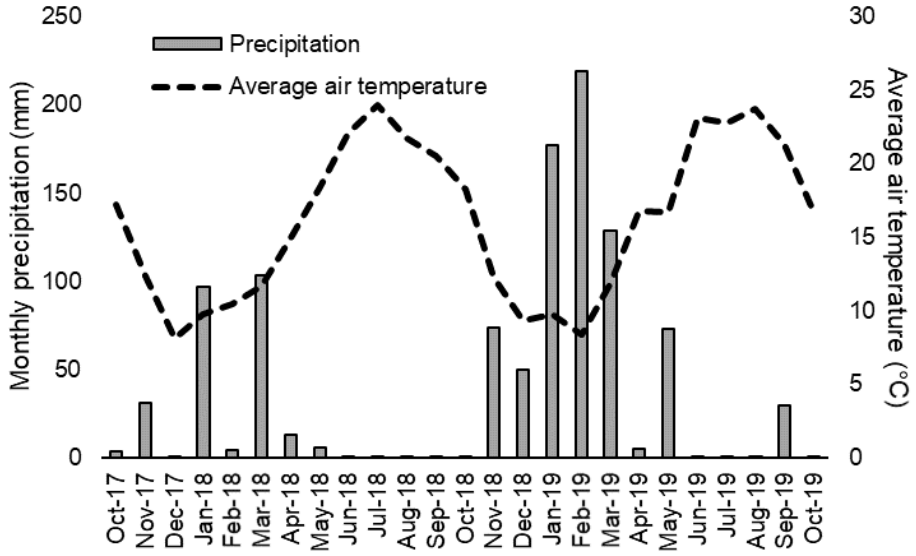

Figure 3.1. Monthly precipitation and average air temperature for Davis, CA from October 2017 through October 2019.

Soil and plant sampling

For each crop in each year, soil samples were taken prior to planting and in the early reproductive phase (early green fruit in tomato and tasseling in corn; Table 3.1). At each sampling date, samples were taken from three locations within each plot, spaced 1.8 m apart within a single bed. At each location, samples were taken 20 cm from the center drip line to a depth of 25 cm. The top 10 cm were discarded, as this portion is not consistently within the wetting zone of these subsurface-drip irrigated beds. In 2018 a 2-cm diameter soil probe was used in all samplings. In 2019 the soil was wetter at sampling and a 4.5-cm diameter Edelman combination auger was used to avoid compaction. About 800 g of soil was obtained from each location, mixed thoroughly, immediately placed on ice, and kept at 4°C until analysis. Different locations were sampled in each year.

Preplant samples were taken on April 4th in 2018 and April 12th in 2019. Due to the difference in operations timing between years, this was prior to composted manure application in 2018 but several months subsequent in 2019. Midseason samplings were timed according to crop phenological stage for each year, crop, and management (Table 3.1). Bulk density samples were taken from the top 0-15 cm at

only one date: just before tomato or corn harvest in 2018, and on July 30th in 2019. Samples were taken 20 cm from the center drip line using a soil corer fitted with a 4.5-cm diameter plastic sleeve.

Soil analyses

Field-moist soils were sieved to 4.75 mm. Gravimetric moisture content was determined by drying a 15-20 g subsample of homogenized soil at 105°C for 24 h. Within one week of sampling, two duplicate samples of 6 g moist soil were weighed into glass 40 ml vials. One set was extracted for 1 hr with 30 ml 0.5 M potassium sulfate (Mulvaney, 1996), filtered through medium retention filter paper (Fisherbrand, Q5), and analyzed for ammonium (NH_4-N) and nitrate (NO_3-N) using colorimetry based on the Berthelot reaction (Forster, 1995; Verdouw et al.,1978) and a single reagent method (Doane and Horwath, 2003), respectively. The sum of these was used to calculate the total mineral N. The duplicate sample was fumigated with chloroform for 24 h and then extracted in the same manner. Organic C was measured in the extracts of fumigated and unfumigated soils using a total organic C analyzer (Shimadzu, Japan). Microbial biomass C was calculated as the difference between C concentrations in fumigated and unfumigated extracts, divided by an adjustment factor k_e of 0.35 (Horwath and Paul, 1996). The remainder of the soil was air-dried and ground to pass through a 2-mm sieve for analyses of additional indicators relating to soil chemistry, organic C pools, biological processes, and physical structure.

Chemical analyses measured on dry soils were electrical conductivity (EC) and pH, measured in a 2:1 water:soil slurry (Thomas, 1996), bicarbonate-extractable phosphorus (P) analyzed colorimetrically (Olsen P; Watanabe and Olsen, 1965), and base cations calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) extracted with ammonium acetate at pH 7 (Helmke and Sparks, 1996; Suarez, 1996) and analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES; Thermo Scientific, Waltham, MA). For indicators relating to organic matter pools, total C and N were measured by dry combustion on soils ground to a fine powder (Nelson and Sommers, 1996). As these soils do not contain carbonates, “total C” will be used to refer to SOC. The particulate organic matter (POM) C and N were assessed using size fractionation to 53 µm followed by dry combustion as described by Cambardella and Elliott (1992). Permanganate-oxidizable C (POXC) was assessed on duplicate 2.5 g samples using the protocol described by Wade et al. (2020).

Two different indicators of biological processes were measured. Rewet CO_2-C was measured as the CO_2-C mineralized over 72 h from 6 g of dried soil rewet to 60% water holding capacity, where water-holding capacity was defined as the water concentration of a saturated soil sample after 1 h free draining in a filter-paper lined funnel (Wade et al., 2016). Water adjustments were made from the top, using a pipet (Wade et al., 2018) and samples were placed in sealed jars fitted with rubber septa for gas sampling and incubated in the dark at 25°C for 72 h. Headspace CO_2-C was measured on an infrared gas analyzer (IRGA; Qubit Systems, Ontario, Canada). As an index of heterotrophic enzyme activity, fluorescein diacetate hydrolysis (FDA) was measured using the method described by Green et al. (2006), with modifications proposed by Prosser et al. (2011). Briefly, three replicate 1-g samples were weighed into 50-ml centrifuge tubes and 30 ml of tris(hydroxymethyl)aminomethane (THAM) buffer (pH 7.6) was added. To two replicates 0.30 ml of FDA solution were added, while an equivalent amount of acetone was added to the third, as a control. Samples were shaken for 3 h on a reciprocal shaker at room temperature. After shaking, the reaction was paused by the addition of 1.2 ml of acetone and briefly vortexing. Samples were let to settle for 10 minutes, after which 1.5 ml was pipetted into 1.5-ml centrifuge tubes and centrifuged at 8800 G for 5 minutes. 1 ml of supernatant was pipetted into cuvettes and measured against a fluorescein standard curve at a wavelength of 490 nm.

Water-stable aggregates and bulk density represented the physical indicators. We determined aggregate stability using a method slightly adapted from that of Kemper and Roseneau (1986). This method was chosen as preliminary tests in our lab showed it to be the least affected by differences in moisture content at sampling and sampling equipment. About 30 g of air-dried soil sieved to 2 mm was placed on top of a 1-mm sieve, and gently shaken to remove the fraction < 1 mm. Exactly 4 g of 1-to 2-mm aggregates were poured in a thin layer on the surface of a 250-µm sieve with a diameter of 6 cm and placed in a clean, dry, 10-cm diameter soil tin. The soil was gradually wet by capillarity by adding deionized water down the side of the tin until the water level just reached the level of the sieve. The sample was let to equilibrate for 15 minutes, after which the water level was brought up and the sample was raised and lowered by hand to height of 1.3 cm for 3 minutes at a rate of 35 oscillations per minute, ensuring that the sieve mesh did not rise above the surface of the water. The can was then placed in a 105°C oven for 24 h to determine the weight of the unstable fraction. To correct for coarse particles, the

sieve containing the stable fraction was transferred to another can containing a solution of 2% sodium hexametaphosphate, in which the remaining aggregates were completely dispersed. The residual sand particles were washed with deionized water and quantitatively transferred to aluminum weigh boats and dried for 24 h at 105°C. The stable aggregate fraction was calculated by subtracting the weight of the unstable and coarse fractions from the initial 4-g sample. To determine bulk density, the height of each 4.5-cm diameter core was measured and the soil was weighed after drying at 105°C for 24 h.

As the texture gradient at the site is not completely addressed by blocking, particle size distribution values measured by ultrasonic dispersion (Gee and Or, 2002) were obtained from Russell Ranch data archives for the top 30 cm of each plot for use as a statistical covariate. Sand was selected to be used as a covariate in statistical analysis, as it differed most widely across the site. In addition, as the sum of sand, silt and clay must equal 100%, sand can also be used as a proxy for the silt + clay fraction, which is known to be important in SOC accumulation (Hassink, 1997).

Statistical analyses

Descriptive statistics for each indicator were generated using PROC UNIVARIATE in SAS (SAS corporation, Cary, NC). The mean, median, standard deviation, skewness, and coefficients of variation (CVs) were assessed for each indicator in each management system across all subsample locations, replicate blocks, crops, dates, and years. As many of the variables showed moderate to extreme skewness, we chose to report the medians and the CVs calculated based on the medians rather than the means (Wade et al., 2018). In most cases the difference between mean- and median-based values was minimal. Outliers were not removed unless values were physically impossible or there was documented evidence of error during the analysis.

Locations within plots were combined to calculate means and CVs for each plot * block * crop * management * date * year combination (n=24). The plot mean values were used to assess the main and interactive effects of management, crop, date and year using analysis of variance in PROC GLIMMIX in SAS. The experiment was analyzed as a crossover design with repeated measures, as detailed in Tao et al. (2015). “Plot” was considered to be the subject, and the two crop sequences (tomato-corn, corn-tomato) considered as sequences of treatments administered over two periods (2018 and 2019), and

"date" was treated as a repeated measure. This approach accounts for the fact that a single subject (plot) received two crop phases, within each of which two measurements were taken in close enough proximity to be dependent (Tao et al., 2015). The crossover design's assumption that carryover effect of crop phase between the two periods is minimal was supported by preliminary exploratory analysis using spaghetti plots for individual plots across dates and years. A compound symmetry (cs) autocorrelation structure was used, as it is often more appropriate for small sample sizes than more complex designs (Shaalje et al., 2002), and preliminary exploration showed it generally gave similar or lower Akaike Information Criterion (AIC) values as more complex designs for most indicators and was more likely to converge. Management, crop phase, sampling date, and year were regarded as fixed. "Year" was treated as a fixed effect, as in the context of this experiment the inference space is the combination of weather, operations timing, sampling equipment, and technician variability which contributed to the difference between the 2018 and 2019 growing seasons, rather than all growing seasons. Three random terms were used: in the first, date was specified as a repeated measure with compound symmetry autocorrelation structure, and the subject defined as plot*crop (Tao et al., 2015). The second, plot within sequence, specifies that the same subject is sampled in two different periods (years). The third, block, allows it to be analyzed as a randomized complete block design. Denominator degrees of freedom were adjusted using the Kenward-Roger method. This method was chosen because of the experiment's relatively small sample size and the potential for unbalanced data due to missing samples, and also because Kenward-Roger is somewhat conservative (Shaalje et al., 2002). Mean separation was performed using Tukey's HSD test with the LINES option in PROC GLIMMIX. The relative strength of a fixed effect was assessed using the adjusted p-values and F statistics. The assumptions of homogeneity of variance and normally distributed residuals around a mean of zero were assessed using Levene's test and visual assessment of residual plots. Indicators were log-transformed as needed to meet assumptions. As the blocking does not entirely account for texture variation across the site, we also performed the same test with sand concentration as a covariate. The covariate was considered to be significant at $p<0.05$.

To assess whether in-plot variability differed between management systems, crop phase, dates, or years, the same procedure was carried out on the CVs of the three locations within each sampled plot. Descriptive statistics were generated for the in-plot CVs using PROC UNIVARIATE.

3.3. RESULTS

Mean differences and variability associated with organic and conventional management

Averaged across locations, plots, crops, dates, and years, values for ORG plots were higher than those for CONV plots in most indicators. Exceptions were pH, extractable Mg, and soil C:N ratio, where CONV exceeded ORG plots, and the POM C:N ratio, where they did not differ (Tables 3.2 and 3.3). The indicator with the largest proportional difference between CONV and ORG systems was mineral N, which was almost 4 times higher in the ORG than CONV plots (Table 3.2). The rate of FDA hydrolysis, MBC, and salinity were all more than twice as high in the ORG than CONV plots. The aggregate stability index was about 20% higher in the ORG than CONV plots. The standard deviation was generally larger for ORG than CONV plots (Table 3.2). However, as median values were also higher in the organic systems, the CVs did not consistently differ between the two systems.

Among the chemical indicators, within the ORG and CONV systems the variability as indicated by CVs was lowest for extractable Ca and Mg, intermediate for K and Na, and very high for mineral N and Olsen P (Table 3.2). Among the different indicators of organic matter pools, POXC and SOC, total N, and C:N ratio varied the least. Microbial biomass C and POM C, N, and C:N ratio had intermediate CVs. The CVs for all biological processes were all relatively high, for both management systems. For the physical indicators, variability was lowest for bulk density and highest for the aggregate stability index.

Table 3.2 Descriptive statistics for soil quality indicators measured in conventional (CONV) and organic (ORG) systems at Russell Ranch. Values calculated for 3 subsamples from 3 replicate blocks, across corn and tomato crops, preplant and mid-season sampling dates and 2018 and 2019 growing seasons (n=72)

	Median		Min		Max		SD		CV (%)‡	
Indicator†	**CONV**	**ORG**	**CONV**	**ORG**	**CONV**	**ORG**	**CONV**	**ORG**	**CONV**	**ORG**
					Chemical indicators					
EC (µS cm^{-1})	96	197	57	121	141	405	19	43	20	22
pH	7.7	7.3	7.3	6.9	8.1	7.8	0.19	0.23	2.5	3.1
Min. N (ppm)	4.9	18	0.68	4.2	9.8	39	2.2	10	45	55
Olsen P (ppm)	34	50	6.7	25	131	96	28	20	83	41
Ca (ppm)	2124	2485	1872	2198	2508	3171	164	190	8	8
Mg (ppm)	1911	1744	1559	1485	2287	2270	183	160	10	9
K (ppm)	171	287	106	173	268	596	36	85	21	30
Na (ppm)	44	85	23	54	77	200	14	20	31	24
					Organic matter pool indicators					
Tot N (%)	0.10	0.15	0.08	0.12	0.11	0.22	0.01	0.02	7.4	10
Tot C (%)	0.93	1.3	0.73	1.0	1.1	1.6	0.09	0.12	10	8.9
Tot C:N	9.5	8.5	8.2	6.8	11.1	9.1	0.45	0.31	4.7	3.6
MBC (ppm)	171	368	90	184	262	617	41	90	24	25
POXC (ppm)	309	465	223	341	391	582	37	56	12	12
POM-N (ppm)	94	181	49	103	158	264	24	32	25	18
POM-C (ppm)	925	1828	468	983	1709	2969	237	364	26	20
POM C:N	10.3	10.1	7.1	7.9	18.9	13.0	1.8	0.9	17	9
					Biological process indicators					
FDA (ppm hr^{-1})	8.8	20.9	4.4	11	20	60	2.5	7.9	28	38
Rewet CO_2 (ppm C)	48	83	16	36	76	169	15	29	32	34
					Physical indicators					
Aggregate stability	0.38	0.46	0.11	0.24	0.63	0.68	0.13	0.10	34	21
Bulk Density (g cm^{-3})	1.35	1.32	1.2	1.1	1.5	1.6	0.07	0.12	5.3	8.8

† EC, Electrical conductivity; Min N, NH_4-N+NO_3-N; MBC, microbial biomass C; POXC, permanganate oxidizable C; POM, particulate organic matter (>53 µm); FDA,fluorescein diacetate hydrolysis rate; Cmin 72=CO_2-C respired from rewet soil after 72 h of incubation.

‡CV calculated on a median basis given the skewed distribution of many of the parameters

Table 3.3. F values and Tukey-adjusted p-values for the main effects of organic or conventional management (Mgt), crop phase of the rotation (corn or tomato), sample date (preplant or mid-season), period within sampling sequence (year 2018 or 2019), and for the interactions of all other effects with management, as well as the effect of Mgt when sand concentration was included as a covariate in the model and for the sand covariate.

Indicator†	Mgt	Crop phase	Sample Date	Period (Year)	Mgt x Crop	Mgt x Date	Mgt x Year	Mgt‡ (sand cov.)	Sand cov.
				Chemical indicators					
EC	339***	47.9***	12.7***	18.8**	0.46	0.88	4.39	297***	0.01
pH	129***	1.72	65.1***	34.2***	1.95	0.75	0.91	124***	0.74
Min N	375***	29.6***	38.5***	425***	0.07	0.51	56.5***	364***	0.06
Olsen P	5.45*	0.29	0.09	35.5***	0.05	0.09	0.54	5.14*	0.45
Ca	43.1***	0.41	9.10**	33.9***	0.06	2.38	2.82	165***	20.8**
Mg	8.9*	0.20	9.35**	0.02	0.09	2.75	3.41	34.1**	16.5**
K	50.8***	2.12	345***	42.7***	2.25	2.47	1.62	66.5***	3.67
Na	251***	9.17*	3.78	0.30	14.7**	2.41	4.56	208***	0.50
				Organic matter indicators					
Tot N	164***	5.27	109***	0.0	0.03	11.3**	0.85	382***	11.9*
Tot C	70.6***	6.51*	128***	3.5	0.32	19.9***	0.49	268***	27.7**
Tot C:N	127***	1.49	3.96	13.3**	2.24	0.01	5.94*	168***	4.07
MBC	222***	3.68	97.7***	12.5**	1.83	2.03	4.91	443***	9.17*
POXC	193	4.90	165***	71.6***	0.51	22.1***	16.5**	384***	9.13*
POM-N	111***	1.21	0.74	8.00*	0.07	12.9**	0.10	218***	10.5
POM C	439***	2.78	2.09	11.2**	0.12	13.7**	0.96	434***	0.80
POM C:N	0.02	0.12	14.6**	0.81	0.00	2.20	0.33	0.12	14.2**
				Biological activity indicators					
FDA	235***	6.60*	123***	55.3***	2.15	2.91	0.44	319***	1.61
Rewet CO_2	268***	0.08	38.8***	128***	0.03	15.5**	12.4**	263***	0.65
				Physical indicators					
Moisture	22.9***	4.39	317***	0.9	0.64	0.09	0.65	21.3***	0.34
ASI	9.47*	1.50	2.0	63.9***	1.99	4.49*	5.25	71.8***	42.8***

*Significant at P<0.05

**Significant at P<0.01

***Significant at P<0.001

† EC, Electrical conductivity; Min N, NH_4-N+NO_3-N; MBC, microbial biomass C; POXC, permanganate oxidizable C; POM, particulate organic matter (>53 µm); FDA,fluorescein diacetate hydrolysis rate; Rewet CO_2=CO_2-C respired from rewet soil after 72 h of incubation.

‡F value and significance level for management when the percent sand was included as a covariate

Other sources of variability and their interactions with management

As assessed by the size of the F value, for most indicators the main effect of management was much larger than the effects of the other sources of variability (Table 3.3). Exceptions were extractable K

and total C, which had stronger main effects for sampling date than for management, and mineral N, Olsen P and aggregate stability, which all had stronger main effects for year than for management. POM C:N, which did not differ between management systems, had larger effects for all three other sources of variability than for management (Table 3.3). While several indicators interacted significantly with management, in every instance the differences between crops, dates, or years differed between management system, while differences between management systems remained constant across levels of the other factors (Table 3.3; Appendix 3 Table 1).

Very few indicators had a significant main effect for crop phase (Table 3.3). These included a strong effect ($p<0.001$) for EC and mineral N and less significant effects ($p<0.05$) for Na, SOC, and FDA. For all these variables, values for soils sampled in the corn phase (that is, prior to corn planting and during corn growth) were higher than soils sampled in the tomato phase (Appendix 3 Table 1). The crop effect was constant across sampling dates and years (Appendix 3 Table 2). In all cases, the main effect for crop phase was small compared with that for management. The only interaction of crop with management was for Na, and was probably due to a single very high outlier in an organic tomato plot in spring of 2019.

The means, medians and variability for selected chemical and physical indicators are shown in Fig. 3.2, and for organic matter pools and biological process indicators in Fig. 3.3. Because crop phase was usually only a minor source of variability the two crop phases are combined for each management by date by year combination.

Most indicators differed significantly between sampling dates (Table 3.3). Only Olsen P, Na, the soil C:N ratio, POM-C, POM-N, and aggregate stability showed no significant main effect for date (Table 3.3). For the chemical indicators, the extractable K was always higher prior to planting than during plant growth for both management systems and years, while the date effect for the other chemical indicators tended to vary depending on the year (Fig. 3.2). Date did not significantly interact with management for any of the chemical indicators (Table 3.3). Mean values for most of the organic matter and biological indicators declined significantly between preplant and midseason for both systems in both years (Fig. 3.3). For POM-C and POM-N, for which date had no significant main effect but had a significant

interaction with management, values were higher in midseason than prior to planting for conventional soils but tended to be higher at preplant than midseason for ORG soils. Soil total C and N, POXC, and the rewet CO_2-C all had a significant management by date interactions, in which the differences between dates tended to be stronger in the ORG soils than in the CONV soils, and the differences between management systems were generally stronger prior to planting than during midseason (Fig. 3.3).

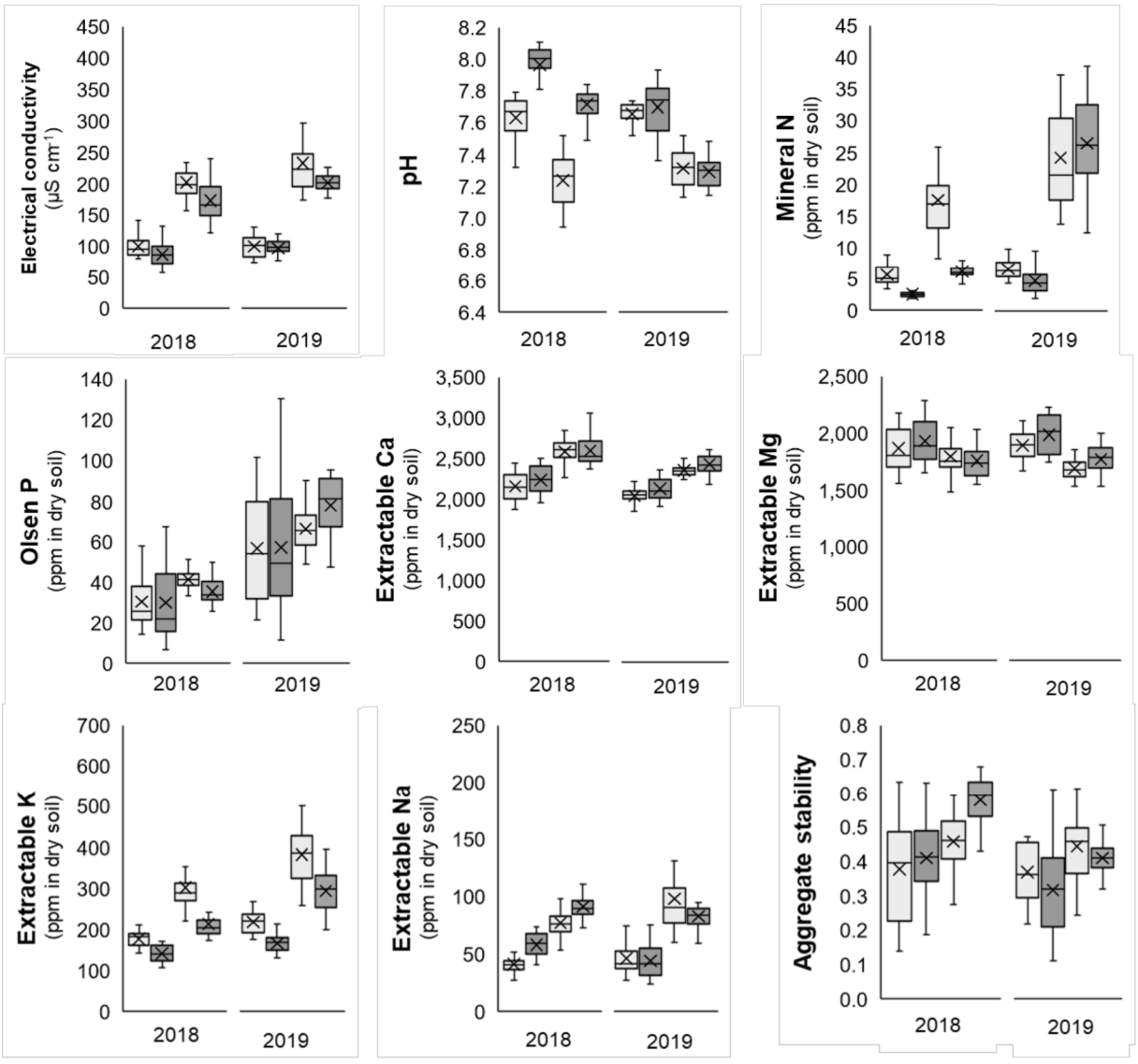

Figure 3.2. Distribution of chemical and physical indicators measured in conventional (gray boxes) and organic (brown boxes) systems, at preplant (lighter boxes) and midseason (darker boxes), in 2018 and 2019. Boxes represent three subsamples taken from two crop phases in three replicate blocks (n=18). Central lines and crosses represent medians and means, respectively. See Table 3.3 for statistical significance of differences.

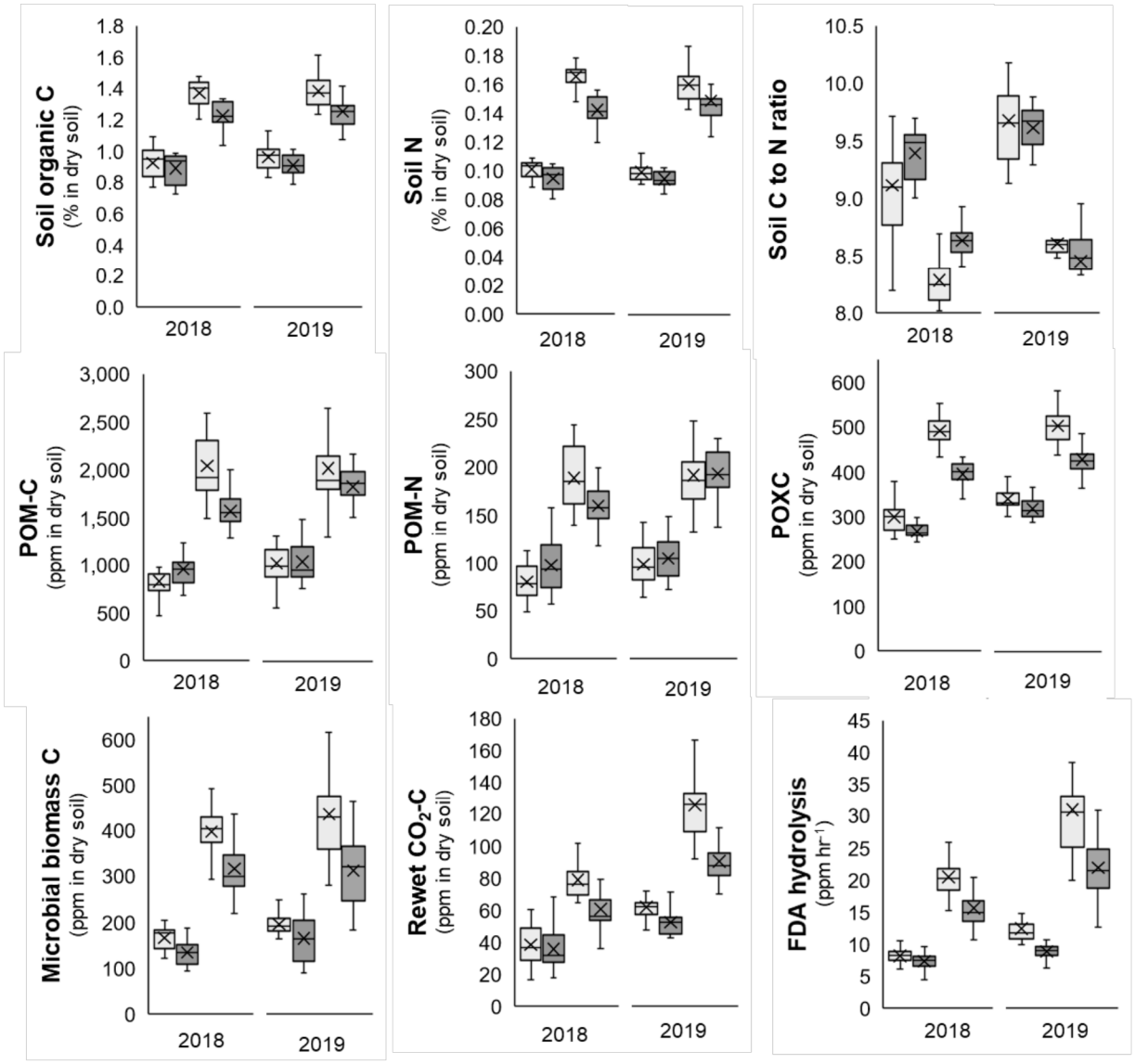

Figure 3.3. Distribution of organic matter and biological process indicators measured in conventional (gray boxes) and organic (brown boxes) systems, at preplant (lighter boxes) and midseason (darker boxes), in 2018 and 2019. Boxes represent three subsamples taken from two crop phases in three replicate blocks (n=18). POM C and N=C and N in particulate organic matter >0.53 µm. POXC=permanganate oxidizable C. Rewet CO_2-C= C measured after 3d incubation from dried soil rewet to 60% water-holding capacity. FDA= fluorescein diacetate hydrolysis rate. Central lines and crosses represent medians and means, respectively. See Table 3.3 for statistical significance of differences.

The bundle of effects encompassed by the period or "year" effect (including weather, operations timing, crop health, and the sampling equipment and technician) was also highly significant for most indicators (Table 3.3). The only factors which did not differ significantly between years were extractable Mg and Na, soil total C and N, and the POM C:N ratio. For the other indicators, higher values were

observed in 2019 than in 2018, except for pH and Ca which were higher in 2018 (Figs. 3.2 and 3.3). In general, these trends were consistent across management systems. Notable exceptions were mineral N and rewet CO_2, in which differences between years were much more significant for ORG than CONV soils (Figs. 3.2 and 3.3), and POXC and POM C:N, in which 2019 values were greater than 2018 values in CONV but not ORG systems (Fig. 3.3).

Effect of a textural covariate on management

To determine the extent to which accounting for the texture differences helped differentiate management systems, all ANOVAs were also run with sand as a covariate. Adding the sand covariate had the greatest effect on the aggregate stability index, increasing the F value 7-fold and lowering the p-value from 0.02 to 0.0004 (Table 3.3). Adding texture also increased F values for most of the organic matter pool indictors. The largest effect was for total C, whose F value increased 4-fold. Sand was also a significant ($p<0.01$) covariate for extractable Ca and Mg among the chemical indicators. The sand covariate was not significant for any of the biological process indicators.

In-plot variability

As indicated by the median CVs of the three subsamples taken within each plot at each sampling, the indicators with the most intrinsic variability were mineral N, Olsen P, and the rewet CO_2-C (Appendix 3 Table 3). These three indicators had median in-plot CVs of 15% or higher, and maximum CVs reaching 40 to 80%. Salinity, Na, aggregate stability, FDA, and the more labile C fractions also had high intrinsic variability, with in-plot median CVs of 10% or higher. Total C and N were the least variable organic matter pool indicators, and Ca, Mg and pH were the least variable chemical indicators, with in-plot median CVs of less than 5%. Analysis of variance performed on the in-plot CVs found that Olsen P and POM C:N ratio were significantly more variable in the CONV system than in the ORG system, and that the ORG system had significantly more variability in extractable K ($p<0.05$; data not shown). Otherwise, intrinsic variability did not appear to differ between management systems.

3.4. DISCUSSION

Developing a useful minimum data set (MDS) and appropriate sampling protocols for soil health assessment requires good regional data about how sensitive different indicators are to management, and

the degree to which they are affected by spatial or temporal variability. In this study, we assessed how strongly several soil health indicators were affected by an organic or conventional management system, and the strength of that signal compared to differences associated with the crop phase within a rotation, sampling date, and year of sampling. We also measured the intrinsic variability of each indicator by analyzing closely spaced subsamples within each plot, and whether adding a texture covariate improved an indicator's ability to differentiate management systems.

Differences between conventionally and organically managed systems

As predicted by our first hypothesis, 25 years under different management had resulted in pronounced differences in the ORG and CONV soils. This is likely due to higher C inputs. A recent analysis of the Russell Ranch Century experiment found that C inputs to the ORG system exceeded those to the CONV system by an average of 67% between 1993 and 2012 (Tautges et al., 2019). Comparison with C stocks measured in the 0-15 cm depth for these sites in 1993, 2003 and 2012 (Kong et al., 2005; Tautges et al., 2019) suggests that C in the plough layer of the ORG system has steadily increased over the 25 years of the experiment's duration, while in the CONV system it has remained relatively stable.

Judging by the magnitude of the system differences as well as the F value, the most sensitive indicators to management were EC, mineral N, and extractable Na among the chemical indicators, the labile organic matter indicator POM-C, and both biological process indicators. The increases in labile C pools, biological activity, and somewhat improved physical structure in the ORG compared to CONV systems are all expected changes in systems which use organic amendments and cover crops (Jian et al., 2020; Lori et al., 2017). Increases in salinity and Na are also frequently observed when manure is used (Miller et al., 2005; Wallingford et al., 1975). However, although on average EC and Na levels in the ORG system were twice as high as in the CONV, they were well below thresholds of agronomic concern for either corn or tomato (Maas and Grattan, 1999). Similarly, increases in available P are also characteristic of manure-fertilized systems (Maltais-Landry et al., 2015). The significantly lower pH in the ORG than CONV systems, however, is unusual; pH is often found to be lower in conventional systems, likely because of the acidification of ammonium fertilizers (Lori et al., 2017). A study which measured C

accumulation in the subsoil at these sites found a disproportionately greater amount of SOC in the subsurface soil of the ORG system compared to the CONV system, suggesting the ORG plots are more leached (Tautges et al., 2019). Higher leaching could help explain the decrease in pH.

Indicator sensitivity to crop phase

It is surprising that crop rotation phase affected so few indicators, as prior to planting the crop phase determines the quality and quantity of last season's residue additions. During the growing season, operations timing, irrigation management and root growth are different between the two crops. Two recent California-wide surveys that measured a variety of soil properties as potential indicators for potential N mineralization concluded that residues may have a strong effect on the relevant pools and processes, especially in soils with a low soil organic matter content, such as those at the study site (Miller et al., 2018; Wade et al., 2016). As corn contributes considerably more residue C than tomato (Tautges et al., 2019) and has higher C:N ratio, the general similarity of most organic matter indicators to crop phase suggests that most were either insensitive to quantity and quality of recent additions or that the responses were transient. The lower EC and mineral N in the tomato phase could both be due to immobilization of mineral N by corn residues, as high NO_3-N can contribute to EC. In addition, processing tomatoes are generally poorer N scavengers than corn (Hills et al., 1983), which may have resulted in higher residual N in the following crop. Interestingly, most of the indicators which were affected by crop phase (mineral N, EC, and FDA hydrolysis) were among those which differed most in magnitude between the ORG and CONV plots. This somewhat supports our hypothesis that the most sensitive indicators will also tend to vary more with non-management sources. However, the fact that in all cases management differences were much stronger than those of either previous or current crop suggests that crop type does not need to be an important consideration in indicator selection in systems with annual crops where residues are incorporated in fall and soil samples are taken the following spring.

Indicator sensitivity to sampling date

Many more indicators were affected by sampling date than by crop phase. Between the preplant sampling and the midseason sampling many changes occur, including the decomposition of labile organic material and release of nutrients as the weather warms, root growth and exudation, changes in moisture

content and distribution as the soil dries and irrigation begins, and eventually depletion of nutrients in the root zone and shading of the soil surface as the crop grows. In addition, in 2018 composted manure was applied between the preplant and midseason sampling. The fact that despite this the organic matter pool and biological process indicators generally decreased between preplant and midseason suggests that the rapid decomposition of labile soil organic material and root nutrient uptake were the most important processes contributing to between-date variability. The idea of a rapid decomposition pulse is also suggested by the lack of crop by date interactions, since the midseason corn sampling occurred later than that of tomato. The tendency for the organic matter pool indicators to decline more sharply in the ORG systems than the CONV systems also suggests they are reflecting cycles of organic matter inputs and decomposition. Interestingly, SOC, total N and POXC, which are thought to represent processed, stable pools (Hurisso et al., 2016), showed very significant date effects. Indeed, when the effect of texture was not accounted for, the strength of the date effect was stronger for total C and N than was management.

Indicator consistency across years

Almost all of the indicators were significantly higher in 2019 than 2018. The two factors which differed most strikingly between the two years were the much higher rainfall prior to the 2019 growing season (nearly triple that prior to 2018) and the fact that compost was applied directly prior to planting in 2018 but the previous fall in 2019. Additional factors which varied between years included a larger cover crop biomass in 2019, the specific locations sampled in each plot, the probe type (necessitated by the wetter soil in 2019), which may have influenced sampling depth, and minor differences in operations timing. Since the year effect was generally consistent across management systems, it appears that the changes in compost application timing and the larger cover crop biomass (which only affected the ORG system) had surprisingly little effect on most indicators. Only mineral N and rewet CO_2-C increased much more strongly in the ORG system than the CONV system between years, suggesting a direct response to the management changes. This finding confirms the idea that rewet CO_2-C is an indicator which quickly responds to practices which increase fertility and thus is often related to N mineralization potential, especially in systems receiving organic N sources (Franzluebbers, 2020; Hurisso et al., 2016).

Notably, total C and N concentrations were among the only indicators which did not change between the years. Together with the variability observed in total C and N between dates, our results are consistent with the idea of a large, stable pool of organic matter. While this is subject to seasonal fluctuations as material is added and decays, overall it is likely to change only slowly (Stott, 2019).

Effect of texture on indicator sensitivity

We hypothesized that adding a texture covariate (sand) would improve the sensitivity of most indicators to management—that is, that soil texture would also be an important determinant for most indicators, and that accounting for this effect, which was not entirely addressed in the blocking, would decrease the within-group variability. Adding a covariate greatly improved the sensitivity of aggregate stability and many of the organic C pool indicators to management, especially total C. This was expected, as finer textured soils are thought to form more stable aggregates and provide more surface area for the long-term protection and stabilization of SOC (Hassink, 1997; Six et al., 2002). However, the only chemical indicators for which adding a texture covariate improved sensitivity to management were extractable Ca and Mg. In this young alluvial landscape, extractable Ca and Mg are both higher in older, more weathered, and finely textured soils. However, the ORG system also receives Ca inputs through the manure compost, while the CONV receives no supplemental Ca. Competition for cation exchange sites probably led to preferential leaching of Mg in the ORG systems and the observed result that ORG had higher Ca but lower Mg than CONV.

Contrary to our hypothesis, however, the texture was not significantly related to most of the chemical indicators, the more labile C pools, or either biological process indicator. Many of these properties were likely directly affected by inputs such as fresh organic matter, fertilizer, or irrigation water, or by exports such as crop uptake. Therefore, the lack of relationship to texture may be because their dynamics are governed mostly by external supply. Another possibility is that their relationship with sand is non-linear. For example, Franzluebbers and Haney (2018), testing rewet C and N mineralization across a range of soil textures, observed the highest rates of rewet respiration in medium textured soils. While examination of our raw data shows that indicators like POM-C and FDA are, in fact, highest in some of

the medium textured soils adding a quadratic covariate did not improve the model fit, suggesting this was not an important mechanism.

Intrinsic indicator variability

The variability between samples taken close together within the same plot on the same date reflects the variation of an indicator over short distances, and how sensitive it is to differences in sampling or analytic techniques. It is important, as it reflects how many samples must be taken or analyzed to get a representative value for the plot. As predicted by our third hypothesis, the smallest in-plot variability was seen in indicators such as total C and N and extractable Ca and Mg, which represent large pedogenic or stable pools. It is worth noting that bulk density was more variable than total C, and thus C stocks, which used both values, were more variable than either. The high in-plot variability of rewet CO_2-C is consistent with the findings of Wade et al., (2018), who noted that this measurement had a high analytical variability compared with other soil tests. The range of CVs for rewet CO_2-C at Russell Ranch, 1.7% to 39% with a median of 15%, is comparable to the range of 13% to 23% reported for this test in rainfed corn in Ohio (Hurisso et al., 2018). The relatively low variability of POXC, another emerging soil health test which is thought to represent a more processed portion of labile C, is also consistent with recent findings that the POXC method is both more repeatable in the field and is subject to less analytical error than rewet CO_2-C (Hurisso et al., 2018; Wade et al., 2018; Wade et al., 2020). The median in-plot CV of 5.4% is comparable to the mean CV of 7- 8% reported by Wade et al. (2020) for samples of different soil orders analyzed in triplicate by several different labs.

The very high variability in available P is surprising, particularly in the CONV system. This contrasts with Hurisso et al. (2018), who observed high analytical precision and spatial correlation on another available P measure, Mehlich P. Subsurface drip-irrigated systems may have higher spatial variability than rainfed systems, as the targeted delivery of small quantities of water and nutrients to a limited area may result in zones of depletion in the wetting zone and high concentrations near the bed edges (Lazcano et al., 2015). The fact that the variability was particularly high in the CONV system suggests that the high variability observed may also partly be due to the fact P is applied in a starter band. As P is relatively immobile in the soil, banding may lead to zones of very high and low

concentrations (Beegle, 2005), and increased sampling density is recommended in fields which receive banded fertilizers (Geisseler and Miyao, 2016).

3.5. CONCLUSIONS

After 25 years of contrasting management, the ORG and CONV systems at Russell Ranch had developed strong differences in almost every indicator tested, particularly in indicators relating to organic matter pools and biological processes. However, the majority of the indicators also declined between preplant and midseason sampling, and were significantly affected by changes in operations timing and weather which occurred between years. Thus, while samples at either date or in either year would be able to differentiate the two management systems, comparison of values obtained in different points in the season or in different years may not, particularly for very sensitive indicators like rewet CO_2-C. Crop phase, however, did not strongly affect most indicators. Taking soil texture into account improved the sensitivity of indicators which represented soil structure, pedogenic nutrients, or relatively processed organic matter pools. With the exception of aggregate stability, these were also the indicators which tended to have the least spatial variability.

Our results have implications for MDS selection and protocol standardization in California and beyond. Given the degree of variability associated with management, soil type, and year observed at a single site in two consecutive years, they underline the necessity of calibrating robust regional thresholds using data from many soil types and management systems and over several years. The strong variability between dates, particularly for biology and fertility-related indicators, confirm the call by Hurisso et al. (2018) for making an effort to sample at the same time each year. As differences between the systems were often higher at the preplant than midseason sampling, preplant sampling appears to be more sensitive as well as more convenient. While the intrinsic variability of rewet CO_2-C and POXC appear comparable to those taken in rainfed midwestern corn, the higher variability in chemical indicators like P suggest that specialized sampling schemes which take into account additional variability introduced over time by practices like fertilizer banding and subsurface drip irrigation may be appropriate. Finally, given the potential for tradeoffs between sensitivity and consistency, including both stable measurements like total C and sensitive measurements like rewet CO_2-C in an MDS may be the best strategy.

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Chapter 4: Relating Soil Health to Crop Performance in Two Irrigated Mediterranean Cropping Systems[4]

ABSTRACT

Accurate assessment of the benefits of soil health building practices to soil function and crop performance requires region-specific data and locally relevant indicators. In this study we used a long-term experiment to measure the effect of 25 years of compost and cover crops on soil health and crop performance indicators in organic and conventionally farmed annual crops in a Mediterranean climate. We measured the strength and consistency of the relationships between several indicators and three functions of a healthy agroecosystem-- carbon (C) storage, net nitrogen (N) mineralization, and crop performance-- over two growing seasons and two crop types. Lastly, we used path analysis to test the hypothesis that healthier soils lead to healthier plants and higher yields. Organic plots had greater C stocks and net N mineralization compared with the conventional plots, but lower yields. The path analysis suggested yields were limited by factors other than N deficiency. The relationships between soil health indicators and soil function were unaffected by crop type but were moderated by yearly changes in weather and operations timing. The indicators most strongly and consistently related to C stocks were permanganate oxidizable C and microbial biomass C, and to N mineralization were CO_2 mineralized from rewet soil and fluorescein diacetate hydrolysis. Our results highlight the importance of including both stable and labile indicators in a soil health assessment. Examination of factors such as pathogen activity, yield stability over time, and potential for input reduction could help link soil health assessments more meaningfully to crop performance.

[4] A version of this chapter has been submitted for publication under the title of "Relating soil health to crop performance in two irrigated Mediterranean cropping systems" to the Soil Science Society of America Journal. As of the date of this writing, it is under review.

4.1 INTRODUCTION

There is widespread interest and considerable government investment in promoting soil health, but little agreement on what soil health means empirically and how to assess it (Wander et al., 2019). Conceptually soil health is defined by its ability to function (Doran et al., 1996); however, soil has many potential functions. The California Department of Food and Agriculture Healthy Soils Action Plan calls for "*investments in outcome-based research programs and activities […] to ensure the most efficient ways to build and maintain soil carbon at the field level while meeting the agronomic needs of growers and ensuring protection of environmental resources*" (CDFA, 2016). Embedded in this statement is the acknowledgment of three critical components which may not always be compatible: building soil carbon (C), meeting crop needs, and protecting the environment. While C sequestration requires long-term C storage in soil fractions or locations that are not biologically available, agronomic productivity requires an actively cycling pool of soil organic matter (SOM) as a source of plant-available nutrients, particularly nitrogen (N), and as a basis for soil biological activity (Janzen, 2006). At the same time, practices like manure addition which build soil C and fertility may also lead to environmental pollution or poor crop yields if they cause excess salinity, sodium (Na), nitrate ($_{NO3}$), or phosphorus (P) (Leytem et al., 2020).

The degree to which a management practice can meet this holistic definition of soil health is strongly context-dependent (e.g. Bowles et al., 2015). Research is therefore needed which can help predict efficient ways to build these different aspects of soil health in a particular site or management system. Such research will depend on indicators which are sensitive to management and consistently relate to different soil health goals, such as C storage and N fertility. However, there is increasing evidence that these indicators need to be regionally selected and calibrated (Caudle et al., 2020; Chahal and van Eerd, 2019; Roper et al., 2017; Zuber et al., 2020). Additionally, the common assumption that improvements in soil health will support higher crop yields or allow reduced inputs may also be management- and region-specific (Miner et al., 2020). In particular, benefits may depend on the degree to which water and fertility are the yield-limiting factors (Leytem et al., 2020). The benefits of "soil health building practices" to crop yields therefore must be assessed in specific contexts (Miner et al., 2020).

There is a particular need for soil health data from Mediterranean climates and for irrigated cropping systems. Most currently used soil health assessment schemes were calibrated for rainfed grain

and pasture crops in humid climates (e.g. Haney et al., 2018; Moebius-Clune et al., 2017). The Mediterranean climate and irrigated, high-value cropping systems which characterize California agriculture are also notably under-represented in national soil health assessment standardization projects (Norris et al., 2020). Given the differing quantities and spatiotemporal distribution of moisture and nutrients in these systems (Schmidt et al., 2018), soil health responses to management and the relationships between indicators and soil function are likely to differ from those observed in humid climates (Leytem et al., 2020). In addition, much of the soil health literature has focused on conservation tillage and cover cropping, but less so on compost use. Compost may be particularly effective at building soil health in Mediterranean climates (Francaviglia et al., 2019), and it is an important practice incentivized under the California Healthy Soils Program (CDFA, 2021).

Long-term research experiments provide a useful way to demonstrate the benefits and tradeoffs of a management practice under relatively stable conditions (Culman et al., 2013; Morrow et al., 2016; Diederich et al., 2019). As noted in a recent meta-analysis, data from long-term field experiments under Mediterranean cropping systems is currently lacking, and is needed in order to elucidate links between management and different aspects of soil health (Francaviglia et al., 2019). Measuring a large set of candidate indicators over time can help detect which indicators are most related to different soil health goals, and how consistent their predictive relationships are between years or under different crops. The results can inform indicator selection for soil health assessment on grower fields (Hurisso et al., 2018).

In this study, C stocks, net N mineralization over 28 d, and yield were chosen to represent three goals of soil health management — C storage, N fertility, and plant performance. The first objective was to demonstrate how these and other indicators have responded to 25 years of a soil health building practice (compost plus cover crop addition) in a long-term experiment in California's Central Valley. The second objective was to identify the indicators that were most strongly associated with the three soil health goals, and to test the consistency of the predictive relationship across years and crops. Finally, we aimed to test the assumption that healthier soils were supporting healthier crops at this site. To accomplish these objectives we monitored several currently used soil health indicators, two in-season indicators of crop performance, and yields in two crops—corn (*Zea mays* L.) and processing tomato (*Solanum lycopersicum* L.)—under organic or conventional management over two consecutive years.

4.2 MATERIALS AND METHODS

Site description

The study was conducted in plots with a corn-processing tomato rotation at the Century Experiment at the Russell Ranch Sustainable Agriculture Facility in northern California (38°32'24"N, 121°52'12"W). In this experiment, a conventional management system using synthetic fertilizer, pesticides, and winter fallow (CONV) is contrasted with a certified organic system with yearly applications of composted poultry manure and a winter legume cover cop (ORG). Detailed site and management information can be found in Tautges et al. (2019) and Schmidt et al. (2018). Briefly, the experiment was laid out in 1993 as a randomized complete design with three blocks. Soils at the site are Rincon silty clay loam soil (fine, smectitic, thermic Mollic Haploxeralfs) and Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Each crop phase of each management system is represented in each block in each year. Both crops are grown on permanent 1.5-m wide beds. Since 2015, all plots have been irrigated by a single subsurface drip line per bed, located down the bed center at a depth of 25 cm. Depth of tillage operations does not exceed 25 cm.

Management and weather during the 2018 and 2019 growing seasons

In the ORG systems, during both years a grass-legume cover crop was seeded the previous November and incorporated in February. In 2018 4 t ha^{-1} of poultry manure compost was incorporated in late April. For the 2019 season the compost was broadcast the previous October on top of the corn and tomato harvest residues, disced, and incorporated. Fertilization in the CONV systems consisted of 350 kg ha^{-1} of an 8-26-6 starter banded at planting, and in-season water-run applications of urea-ammonium nitrate (UAN 32) to yield an annual total of 235 kg N ha^{-1} in corn and 200 kg N ha^{-1} in tomato. Tomatoes were planted in late April in both years and systems, and mechanically harvested in late August to early September. CONV corn was seeded in late April or early May. ORG corn was planted in late May 2018 and early June 2019. Both systems in both years were mechanically harvested in October.

Rainfall prior to the 2018 growing season (October through March) was 240 mm and prior to the 2019 growing season was 650 mm (California Irrigation Management Information System). Average air

temperatures were generally similar between years. February and May were on average about 2°C cooler in 2019 than 2018, and April was about 2°C warmer.

Soil and plant sampling

For each crop in each year, soil samples were taken in early April, prior to planting. Due to the difference in operations timing between years, this was prior to compost application in 2018 but several months subsequent in 2019. Samples were taken from three locations within each plot, spaced 1.8 m apart within a single bed. At each location, samples were taken 20 cm from the center drip line to a depth of 25 cm. The top 10 cm were discarded, as this portion is not within the wetting zone of these subsurface-drip irrigated beds during the growing season. Thus, it may not represent the zone of maximum biological activity or root uptake. Bulk density samples were taken in midseason in both years with a 4.5-cm diameter core sampler.

Chlorophyll fluorescence (CF) and leaf N were measured twice during the growing season: at early growth (V5 stage for corn and early flowering for tomato; hereafter “early season”) and during rapid plant growth and N uptake (tasseling stage in corn and early green fruit stage in tomato; hereafter “midseason”). Each measurement was performed at each location on a healthy, recently matured leaf, either measured or collected in the early morning. Leaves taken from each location for N analysis were composited and dried at 60°C. Mechanically harvested corn and tomato yields for each plot were provided by the Russell Ranch staff.

Soil and plant analyses

We determined ammonium (NH_4) and NO_3 by colorimetric methods and microbial biomass C by the chloroform fumigation extraction method on sieved field-moist soil as described in Geisseler et al. (2009). The remainder of the soil was air-dried and ground to pass through a 2-mm sieve for analyses of additional indicators relating to soil chemistry, organic C pools, biological processes, and physical structure. Soil EC and pH were determined in 2:1 water to soil slurries (Thomas, 1996), and base cations and Na were measured in neutral ammonium acetate extract (Helmke and Sparks, 1996; Suarez, 1996). Finely ground samples were analyzed for total C and N by dry combustion (Nelson and Sommers, 1996). Particulate organic matter C and N (POM-C and POM-N) were assessed by size fractionation to 53 µm

followed by dry combustion (Cambardella and Elliott, 1992). Permanganate-oxidizable C (POXC) was assessed with the protocol described by Wade et al. (2020b). A wet aggregate stability index (ASI) was measured as the proportion of air-dried 1-2 mm aggregates remaining after 15 minutes slaking and 3 minutes wet sieving at 35 oscillations per minute (Kemper and Roseneau, 1986). To determine bulk density, the height of each 4.5-cm diameter core was measured and the soil within the core was weighed after drying at 105°C for 24 h. Carbon stocks were calculated using the mean bulk density for the three locations over two years as the average bulk density for each plot. As the texture gradient at the site is not completely addressed by blocking, particle size distribution values measured by ultrasonic dispersion (Gee and Or, 2002) were obtained from Russell Ranch data archives for the top 30 cm of each plot for use as a statistical covariate.

Potential enzyme activity and respiration from rewet soil were used as biological process indicators. As an index of heterotrophic enzyme activity, fluorescein diacetate hydrolysis (FDA) was measured by a method adapted from Green et al. (2006), with modifications proposed by Prosser et al. (2011). The method is fully described in Miller et al. (2019). Respiration was measured from 6 g of dried soil rewet to 60% water holding capacity, where water-holding capacity was defined as the water concentration of a saturated soil sample after 1 h free draining in a filter-paper lined funnel (Wade et al., 2016). Water adjustments were made from the top, with a pipet (Wade et al., 2018) and samples were placed in sealed jars fitted with rubber septa for gas sampling and incubated in the dark at 25°C. Headspace CO_2-C was measured on an infrared gas analyzer (IRGA; Qubit Systems, Ontario, Canada). As proposed protocols for respiration from rewet soil use several different incubation times, measurements were made after 24 hr (Cmin-1d), 3 d (Cmin-3d), and 28 d (Cmin-28d). Following 28 d of incubation, samples were extracted with K_2SO_4 and analyzed for mineral N. Duplicate samples of dried soil were also extracted and analyzed, and net N mineralization over 28 d (hereafter N mineralization) was calculated as the difference between mineral N in the dried and incubated soil. Net N mineralization over 28 d of moist aerobic incubation is closely related to longer term N mineralization, and is used here as a proxy for N mineralization potential (Franzluebbers, 2020; Wade et al., 2016).

Chlorophyll fluorescence was measured with a HandyPea continuous excitation fluorimeter (Hansatech Instruments, UK). The Fv/fm parameter, a measure of photosynthetic efficiency, was used to represent general plant stress. It has been shown to be sensitive to abiotic stresses including nutrient deficiency, salinity, and drought (Kalaji et al., 2016). Lower numbers indicate more stressed plants. Leaf N concentrations were determined on dried and ground samples by dry combustion.

Statistical analyses

Locations within plots were combined to calculate means for each plot * block * crop * management * year combination (n=24). The plant measurements for each plot in each year were normalized to the maximum value for the respective crop types over the two years of the study. All subsequent analyses were done on the plot means for the soil indicators and normalized plot means for the yields.

Underlying sources of variability within the dataset were characterized by principal components analysis with PROC PRINCOMP in SAS (SAS Corporation, Cary, North Carolina). The first five principal components were selected based on visual inspection of the scree plots. The main and interactive effects of management, crop, and year were analyzed for the three soil health goals (C stocks, net N mineralization, and yield) by analysis of variance in PROC GLIMMIX in SAS. Management, crop, and year were regarded as fixed and block as random effects, and sand was included as a covariate. The experiment was analyzed as a crossover design, with an additional random term of plot nested within cropping sequence (Tao et al., 2015). "Plot" was considered to be the subject, and the two crop sequences (tomato-corn, corn-tomato) considered as sequences of treatments administered over two periods (2018 and 2019). This approach accounts for the fact that a single subject (plot) received two crop phases (Tao et al., 2015). Assumptions were tested with Levene's test and visual assessment of residual plots. Indicators were log-transformed as needed to meet assumptions.

The consistency of the predictive relationships between the three soil health goals and a subset of eight indicators which have been recommended as sensitive to management and relevant to soil function (Stott, 2019) were assessed through simple linear regressions using PROC REG in SAS. The slopes, adjusted r^2 values, and significance of these relationships were assessed separately for the

different years or crops, averaged across all other factors. Slopes obtained for different years or crops were compared with PROC GLM in SAS.

A confirmatory path analysis model was developed to test the hypothesis that soils with high organic matter (C stocks) would have greater N fertility (N mineralization), leading to better N nutrition (midseason leaf N) and less stressed plants (midseason CF) which in turn would produce higher yields. The "piecewiseSEM" package in R was used (Lefcheck, 2016). The model had 5 parameters to test n=24 observations, slightly violating the rule that the ratio of parameters to observations should exceed 5:1 (Lefcheck, 2016). However, tests with a smaller number of parameters yielded similar parameter estimates to the larger model, suggesting the violation did not affect estimate stability. To account for the fact that measurements were made on the same plot over two years under different crops, piecewise tests were run as multilevel mixed models, with plot nested within cropping sequence as a random term (Shipley, 2009). Prior to analysis soil data was normalized to the highest value in the dataset, and plant data to the highest value for the respective crop. All data were log-transformed prior to analysis, as the residuals from the ANOVAs suggested this improved linearity. The residuals from the path analysis were tested for multivariate normality with Mardia's test and by examination of the qq plot with the "MVN" package in R (Korkmaz et al., 2019).

4.3 RESULTS

Relationships and sources of variability among soil and plant health indicators

Relationships among soil and plant health indicators were summarized by principal components analysis (Fig. 4.1). The first principal component (PC1) strongly separated the two management systems and slightly separated years, and accounted for more than half of the variability in the dataset. All of the organic C and biological process indicators had approximately equal positive loadings on PC1, while yields had a negative loading. All indicators loading positively on PC1 had strong positive correlations to each other and negative correlations with yield (Appendix 4 Table 1). The chemical indicators EC, K, Na, P, Ca, and mineral N also had high positive loadings on PC1. The second principal component (PC2) accounted for 18% of the variability and the most important eigenvectors were the soil texture components (Fig. 4.1). Magnesium and soil aggregate stability, both of which were strongly correlated

with soil texture, also loaded highly on PC2. The third principal component (PC3) accounted for 9% of the variability and was higher for all 2019 samples than 2018 samples. Fourth and fifth principal components (PC4 and PC5), accounting for 5% and 3% of the variability, were highly loaded for midseason leaf N and early season CF respectively.

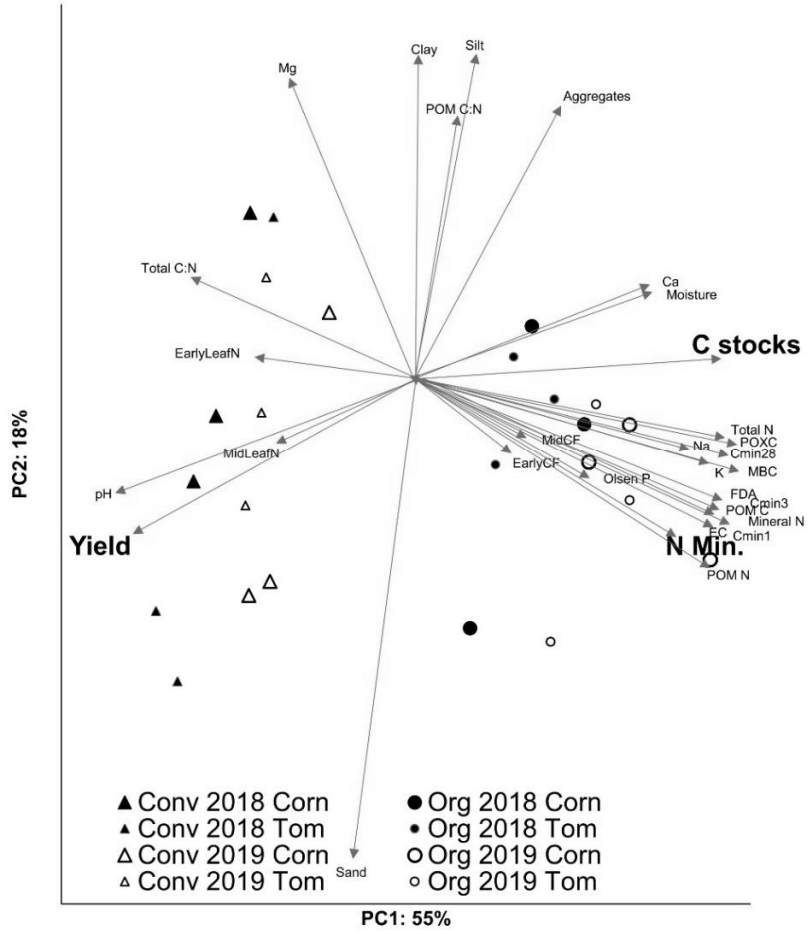

Figure 4.1. Principle components analysis of soil health indicators for samples taken from corn and tomato plots under conventional or organic management in 2018 and 2019. Values for plant indicators were normalized to the maximum value for the crop. EC, electrical conductivity; MBC, microbial biomass C; POXC, permanganate oxidizable C; POM, particulate organic matter; Nmin-28d, net N mineralization from rewet soil over 28d aerobic incubation; FDA, fluorescein diacetate hydrolysis; Cmin-1d, 3d, 28d, CO_2-C measured from rewet soil after 1, 3, and 28 d of aerobic incubation; ASI, aggregate stability index; Early and Mid leaf N, N concentration in a recently matured leaf during early and midseason growth; Early and Mid CF, chlorophyll fluorescence in a recently matured leaf during early and midseason growth

System differences in soil health and plant yields

Over both years, C stocks in the 10-25 cm depth averaged 18.9 Mg ha^{-1} in the CONV plots and 23.2 Mg ha^{-1} in the ORG plots. The N mineralized over 28 days averaged 18 mg kg^{-1} soil in the CONV plots and 41 mg kg^{-1} in the ORG plots. Both differences were highly significant (Table 4.1). The C stocks were similar in 2018 and 2019; however, N mineralization differed strongly between years, being higher in 2019 than 2018 for both ORG and CONV systems (Table 4.1; Appendix 4 Table 2). The sand content was a highly significant covariate for C stocks but not N mineralization (Table 4.1). ORG corn grain yields, 10 Mg ha^{-1} in both years, were significantly lower than those in the CONV system (14 and 17 Mg ha^{-1} in 2018 and 2019 respectively). The county average for both years was 12 Mg ha^{-1} (USDA QuickStats). The CONV tomato yields of 99 and 102 Mg ha^{-1} in 2018 and 2019 were similar to the county average of 103 Mg ha^{-1}; while the ORG yields of 51 and 71 Mg ha^{-1} for the two years were significantly lower than the CONV yields. Across both crops and management systems yields were higher in 2019 than 2018; however, a significant interaction between crop, management and year showed that yield increases between 2018 and 2019 were only significant for conventional corn and organic tomato (Table 4.1).

Table 4.1: F-values for comparisons of the main effects of management (conventional or organic), year (2018 or 2019), and crop (corn or tomato), and their interactions. Yields for corn and tomatoes were standardized separately to the highest corn or tomato yield value in the dataset, such that the "Crop" effect tests distribution rather than absolute yield.

	C stocks	Mineralizable N	Yield (standardized)
Mgt	251.12***	210.10***	89.73***
Year	1.18	902.67***	8.1*
Mgt*Year	0.53	17.15**	0.13
Crop	0.17	0.62	1.7
Mgt*Crop	0.76	1.17	0.64
Year*Crop	0.02	3.99	0.89
Mgt*Year*Crop	0.35	0.73	5.46*
Sand	34.67***	0.04	0.00

* Significant at p<0.05 **Significant at p<0.01 ***Significant at p<0.001

Relationships between indicators and goals for different years and crops

To determine the strength and consistency of the predictive relationships between indicators and soil function, we conducted linear regressions between C stocks or N mineralization and eight of the

measured indicators which were recommended in a recent NRCS Technical Note (Stott, 2019) as being associated with functionally relevant soil processes: namely total N, POXC, POM-C, MBC, Cmin-28d, Cmin-3d, FDA (as an index of enzyme activity), and aggregate stability (Figs. 4.2 and 4.3). The C mineralized from rewet soil over 24 hr (Cmin-1d) is also a recommended indicator; however, it behaved similarly to the Cmin-3d but was more variable and so was not included. Regressions were run separately for years and crop phases. Only years are presented, as crop phase did not affect the relationship of any of the indicators with C stocks or N mineralization.

The slopes of the linear regression between C stocks and the organic matter pool indicators and Cmin-28d did not differ significantly with year (Fig. 4.2a-e). Conversely, the slope of the linear relationships was 70% higher in 2018 than 2019 for Cmin-3d ($p=0.04$) and 90% higher for FDA hydrolysis ($p=0.01$), as both Cmin-3d and FDA increased in 2019 but C stocks did not (Fig. 4.2f-g). The most consistent predictors of C stocks between years were soil N, POXC, and MBC. The r^2 of the relationships between these indicators and C stocks exceeded 0.85 in both years.

As with C stocks, all indicators except aggregates had highly significant relationships with N mineralization in both years (Fig. 4.3). However, the slopes were significantly ($p<0.01$) higher in 2019 than 2018 for all indicators, as N mineralization increased more in 2019 than any of the predictor indicators. The increase in N mineralization was especially pronounced in the ORG treatment. The indicators whose between-year changes most closely reflected those of N mineralization were FDA hydrolysis (Fig. 4.3g; 67% higher slope in 2019 than 2018) and Cmin-3d (Fig. 4.3f; 109% higher slope). The indicator which was least responsive to the between-year change in N mineralization was POXC, for which the increase in N mineralization per unit POXC increase was 250% higher in 2019 than 2018.

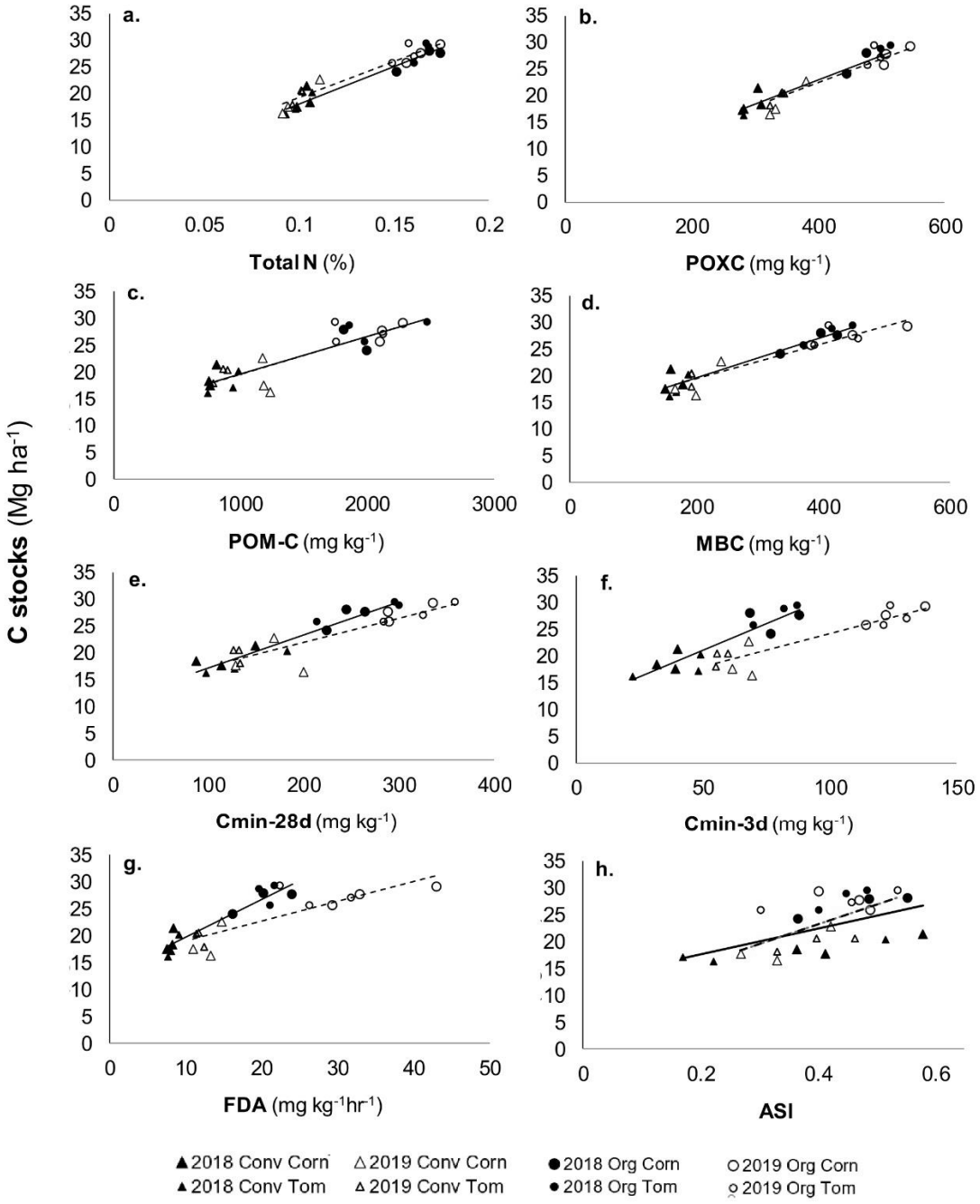

Figure 4.2. Relationship between C stocks and a) total soil N; b) permanganate oxidizable C (POXC); c) Particulate organic matter C (POM-C); d) Microbial biomass C (MBC); rewet CO_2-C respired after incubation at 25°C and 60% water-holding capacity for e) 28 d (Cmin-28d) and f) 3 d (Cmin-3d); g) fluorescein diacetate hydrolysis rate (FDA); and h) aggregate stability index (ASI). Conv=Conventional, Org=Organic, Corn= corn was planted in the sampling year and Tom=tomato was planted in the sampling year. Solid lines represent the linear relationship for 2018 and dotted lines the linear relationship for 2019.

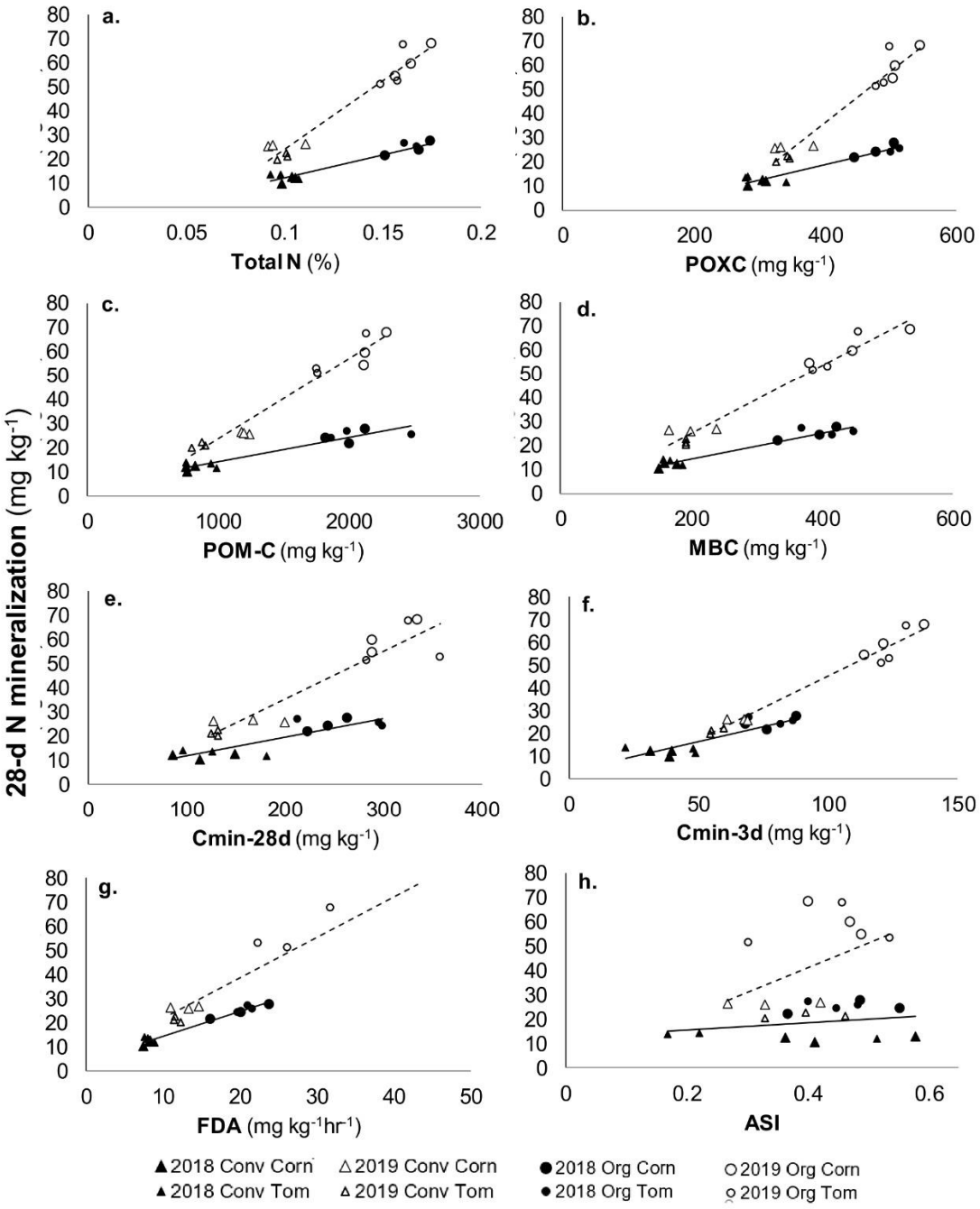

Figure 4.3. Relationship between net N mineralized over 28 d of incubation at 60% WHC and a) total soil N; b) permanganate oxidizable C (POXC); c) Particulate organic matter C (POM-C); d) Microbial biomass C (MBC); rewet CO_2-C respired after incubation at 25°C and 60% water-holding capacity for e) 28 d (Cmin-28d) and f) 3 d (Cmin-3d); g) fluorescein diacetate hydrolysis rate (FDA); and h) aggregate stability index (ASI). Conv=Conventional, Org=Organic, Corn= corn was planted in the sampling year and Tom=tomato was planted in the sampling year. Solid lines represent the linear relationship for 2018 and dotted lines the linear relationship for 2019.

Relationships between soil health and crop performance

Most soil health indicators were significantly negatively related to yields across both crops and years ($p<0.001$; Appendix 4 Figure 1). However, the relationships between indicators and yields were not significant when compared within either management system.

Confirmatory path analysis (Shipley, 2009) was used to test the hypothesis that high soil organic matter and N fertility would lead to result in healthier plants that would produce higher yields (Fig. 4.4).

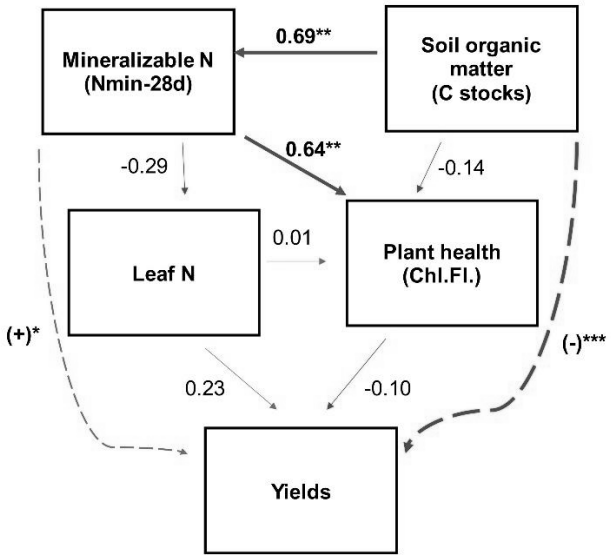

Figure 4.4. Confirmatory path analysis testing the hypothesis that soils with higher organic matter (C stocks) and greater N fertility (N mineralized over 28 d in an aerobic incubation at 60% water holding capacity) will lead to better N nutrition (Leaf N) and healthier plants (higher chlorophyll fluorescence Fv/fm during rapid growth), that in turn will lead to higher crop yields. Analysis showed a poor fit, with a Fisher's C of 25 ($p<0.0001$), and d-sep tests which indicated significance of missing paths between yields and C stocks or N fertility (dotted arrows). Numbers are parameter estimates for variables normalized to the highest value in the dataset and log-transformed prior to analysis. Blue denotes a positive and red a negative relationship, and larger arrows denote stronger relationships. Asterisks *, **, and *** represent p-values of <0.05, 0.01 and 0.001, respectively.

Measures of plant health included both a general indicator of plant photosynthetic efficiency (CF; that is, the Fv/Fm parameter of chlorophyll fluorescence) and leaf N, a specific measure of N nutrition, both measured during the early reproductive phase for both crops. The path analysis showed a strong

contribution of SOM to mineralizable N, and a positive effect of mineralizable N on CF (Fig.4.4). However, the path between mineralizable N and leaf N was non-significant. There was no relationship between leaf N and CF. The directional separation (d-sep) test of the direct path between N mineralization and yields was slightly significant and positive, while that between C stocks and yields was highly significant and negative (Fig. 4.4). In confirmatory path analysis, a significant d-sep test indicates a poor relationship between the model and reality due to the presence of important missing factors (Shipley, 2009). This result therefore indicates the presence of an unmeasured factor or factors which had a strong but inverse relationship to both C stocks and yields, and of one which had a slightly significant relationship to both N mineralization and yields.

4.4 DISCUSSION

Long-term organic management increased soil health but not plant performance

Our first objective was to demonstrate how soil and plant health indicators responded to 25 years of a soil health building practice. The principal components analysis suggested four independent sources of variation in the dataset: management, soil type, yearly differences in weather and operations timing, and unrelated factors affecting crop performance. By far the most important source of variation was PC1, which likely reflected differences in the quality and quantity of organic matter additions. The high loadings of chemical indicators such as EC, K, and Na indicate that long-term compost use contributed to buildup of salts as well as labile organic matter in the ORG systems (Grijalva et al., 2010; Leytem et al., 2020). However, values for salinity, pH, and sodium were all in ranges considered to be non-limiting for crop production (Appendix 4 Table 2), suggesting that these factors were likely not the primary reasons that yields had negative loadings. There was substantial textural variability at the site, with sand contents ranging from 13-32%. Despite this, texture differentiated sites to a much lesser degree than management. The high loadings of aggregate stability, Mg and the POM C:N ratio on PC2 suggest that at these sites they were a function of intrinsic edaphic factors rather than of management. Our results are in line with the global meta-analysis of Francaviglia et al. (2019) which concludes that in Mediterranean climates C inputs appear to be the main driver of C accumulation, with soil texture having a more minor role.

The variability between years appears to have been principally moderated through organic matter quality and quantity. All plots in 2019 were higher on PC1 than their components in 2018, while PC3, which separated observations only by year and likely reflects random differences in weather, operations timing or sampling between the years, represented only a small part of the dataset variability. The fact that leaf N and CF accounted for only a small part of the dataset variability and did not load alongside yields or other indicators also indicates that whatever limited yields in the ORG system was not directly related to N nutrition or photosynthetic efficiency during early and midseason growth.

The ANOVAs confirm that ORG management significantly increased C stocks and N mineralization, indicating a pronounced improvement in soil health. These results are in line with a recent meta-analysis of Mediterranean cropping systems which found that for arable systems compost addition was a highly effective practice for increasing C storage (Francaviglia et al., 2019). The 23% average increase of C stocks in the ORG compared to CONV plots at Russell Ranch is very close to the increase of 23.5% reported worldwide for organically versus conventionally fertilized crops in Mediterranean systems (Aguilera et al., 2013). The C stocks were significantly affected by texture but not year, while N mineralization differed greatly between years but was not related to texture. This is interesting, given the important differences between the two years—compost was applied after the 2018 sampling but prior to the 2019 sampling, the rainfall preceding the 2019 season was almost triple that preceding the 2018 season, and cover crop biomass was higher in 2019. Together, these results indicate that while both goals were significantly affected by management history, texture was a stronger control on C stabilization and climate and recent management were stronger controls on N mineralization (Lavallee et al., 2019; Wade et al., 2016). Our results demonstrate that a quarter century of compost and cover crop additions have built substantial pools of both stable and actively cycling soil organic matter in the ORG systems (Kong et al., 2005; Tautges et al., 2019). However, improvements in soil health were not reflected in crop yields. The reasons for this are explored in detail below.

Consistency of relationships between indicators and goals for different years and crops

Soil health indicators which will be used for monitoring the efficacy of a practice over time must have consistent predictive relationships with the property of interest. In pursuit of our second objective,

we conducted linear regressions between C stocks or N mineralization and eight soil process indicators, and tested the tested the similarity of the slopes across years and crop types. Total N, POXC, POM-C, MBC, Cmin-28d, whose relationships to total C were similar between the two growing seasons, were relatively unaffected by the climate and management differences between years. Although the latter four assays are thought to measure relatively labile C fractions, they appeared to be primarily influenced by the stock of stable organic matter built up over the years. The closest and most consistent predictors of C stocks between years were soil N (which, as it also relies on the change in the total SOM pool, has similar limitations to total C as an indicator of soil quality change), POXC, and MBC. This result is in line with studies which suggest that POXC represents a relatively processed fraction of the organic matter pool whose behavior closely mirrors that of total SOM (Jagadamma et al., 2019; Morrow et al., 2016).

Although the biological process indicators Cmin-3d and FDA hydrolysis were correlated strongly with C stocks within each year, their variable relationships to C stocks in different years suggests they represent only the most labile organic matter fractions and are unreliable indicators of total C accumulation. This would be in accordance with several studies which found that C mineralization was not always related to total C (Caudle et al., 2020; Hargreaves et al., 2019; Laffely et al., 2020).

The pattern was reversed for N mineralization. The FDA hydrolysis activity and Cmin-3d had the most consistent relationships to potential N mineralization across the years of any indicator, and POXC the most variable. This tradeoff is reasonable, as by definition more stable organic matter fractions are less likely to respond to the fluctuations in climate and input quality which affect N mineralization rates (Hurisso et al., 2016; Janzen, 2006). Overall, our results demonstrate that including a combination of labile and stable indicators will allow for a more reliable assessment of the C sequestration and turnover facets of soil health (Hurisso et al., 2016). Additionally, the strong yearly fluctuations suggest that if biological process indicators such as Cmin-3d or FDA are used in soil health assessments, identification of “healthy” ranges for a particular region and soil type will require several years of data.

Relationships between indicators and soil health goals were similar between crop phases. This interesting, as the quantity and quality of residues from the previous year’s crop differs strongly between

corn and tomato (Tautges et al., 2019). It suggests that neither the quality nor quantity of the previous year's crop residues was an important mediating factor between soil process indicators and function.

Soil health did not predict crop performance

Did healthier soils cause poor yields? Probably not, given the lack of relationship between yields and indicators within management systems. The overall negative correlations were more likely due to the ORG plots having higher indicator values and lower yields than the CONV plots, rather than any intrinsic negative effect of organic matter on yields. The inverse relationship is not unexpected, as on average globally organic systems tend to have healthier soils than conventional systems but yield less (Seufert and Ramankutty, 2017). The yield gaps between conventional and organic systems in industrial agriculture are often attributed to nutrient limitation, especially of N (Connor, 2008; de Ponti et al., 2012). However, the plant performance indicators and the results of the path analysis suggest that N was not the primary limiting factor at the Century Experiment in the years of our study. The significant positive relationship between C stocks and N mineralization indicates the cycling of the OM built up over the years. The lack of relationship between N mineralization (which was much higher in the ORG system) and leaf N (which did not differ on average between the two systems) suggests that soil N mineralization in the ORG plots and fertilization in the CONV plots did not differ substantially in their contributions to crop N nutrition. The lack of relationship between leaf N and CF, along with the fact that values for leaf N were within published sufficiency ranges for corn and tomato in both systems, are in line with the idea that N was not generally limiting to plant growth. Given that N mineralization and CF both tended to be higher in 2019 than 2018, the direct significant relationship may be due to some extraneous environmental factor which affected both.

The highly significant d-sep tests indicate that important factors relating C stocks and N mineralization to crop yields were not included in the model (Lefcheck, 2016). The missing factor with a strong but inverse relationship to C stocks and yields likely relates to disease pressures. During the two years of the study ORG corn yields were reduced by fusarium ear rot, and in 2018 ORG tomato yields were heavily reduced by a complex of diseases, including crown rot, bacterial canker, and fusarium wilt (Cassandra Swett, personal communication). These diseases only became severe in the second half of

the season, and so did not affect the CF measurement. The positive path between N mineralization and yields once the separate effects of C stocks were accounted for were likely related to the between-year weather differences which resulted in higher values of both.

Our findings contrast with those of Culman et al. (2013), who observed that labile C and N fractions had a significant positive relationship with rainfed corn yields in Michigan. However, they are in line with several recent studies which suggest that most soil health assessment strategies may only be related to crop yield if soil water availability or fertility are the limiting factors (Leytem et al., 2020; Miner et al., 2020; Zebarth et al., 2019). Healthy soils can benefit crops by supplying nutrients and improving water infiltration and retention (Stewart et al., 2018). However, in fertilized and irrigated cropping systems, these benefits often do not result in higher yields (Miner et al., 2020). To better evaluate the benefits of soil health, a comparison of the inputs (i.e. fertilizers, pesticides, and irrigation water) needed to achieve yields comparable to fields with a lower soil health score may be more informative. For example, a recent study on rainfed corn using N rate trials across the Midwestern Corn Belt found that soils with higher soil biological health scores tended to achieve equivalent yields at lower N rates than less "healthy" soils at the same sites (Wade et al., 2020a).

Our results also suggest that including aspects of soilborne disease incidence or resistance in a minimum dataset may help soil health assessment to more reliably relate to crop productivity, especially in high-input systems where water and nutrients are less likely to be limiting (Van Bruggen and Semenov, 2000; van Bruggen et al., 2019). While pests and pathogens are explicitly mentioned in some definitions of soil health (FAO, 2008.; Kibblewhite et al., 2008), most soil health assessment schemes do not include indicators which directly relate to either (e.g. Moebius-Clune et al., 2016; Norris et al., 2020; Stewart et al., 2018; Stott, 2019). Such indicators could include assays for the presence of a target pathogen under different experimental conditions, or the resilience of some soil function to an experimental inoculation or invasion (Tubeileh and Stephenson, 2020; Van Bruggen and Semenov, 2000).

By definition a healthy soil supports crop production; however, one year's agronomic performance is not synonymous with soil health (Wander et al., 2019). Resilience metrics such as yield stability over time and the ability to withstand shocks may better link soil health to crop productivity as they directly

address the sustained ability of the soil to function, especially under changing climactic conditions (Gaudin et al., 2015; Nouri et al., 2020). The few studies which compare yield resilience between organic and conventional systems are conflicting, and suggest the effects are likely crop- and duration-dependent (Seufert and Ramankutty, 2017). An analysis of yield resilience in the Century Experiment at Russell Ranch from 1993 through 2017 found that average ORG processing tomato yields did not differ from those in the CONV system, and had greater stability and a lower probability of crop failure (Li et al., 2019). Conversely, for corn during the same period ORG yields were consistently lower than CONV yields, and were also less stable and had a higher probability of crop failure. Thus, yield stability analysis suggests that from 1993 to 2017 the ORG soil interacted with other yield-influencing factors in a way that was "healthier" for tomato than for corn. For example, Li et al. (2019) speculate that the timing of N availability in the ORG system may have matched the needs of tomato, which takes up relatively little N during early vegetative growth, but not of fast-growing corn. Additionally, the fact that in 2018 and 2019 the ORG but not CONV tomato yields were lower than in any of the previous five years (50 and 71 Mg ha^{-1}, respectively, compared to the 2013-2017 average of 94 Mg ha^{-1} reported by Li et al., 2019), indicates that the effects of management on yield resilience may change over the years (Nouri et al., 2020). For example, if 2018-2019 ORG tomato yields were low because soilborne pathogen populations had built up over time in the ORG soils, yield resilience could be expected to continue to decline. Including yield resilience metrics in a soil health assessment may be useful for identifying crop-specific effects of management on soil health when combined with other measures (Miner et al., 2020; Seufert and Ramankutty, 2017).

4.5 CONCLUSIONS

According to a wide range of chemical, biological, and physical indicators, 25 years of compost and cover crop addition had dramatically improved soil health and fertility in this drip irrigated annual cropping system, resulting in both higher C stocks and N mineralization. POXC and MBC were the labile SOM fractions which most consistently predicted C storage, while FDA hydrolysis and short-term C respiration most consistently predicted N mineralization. Monitoring schemes which aim to use simple assays to help growers assess how well a practice is fulfilling multiple soil health goals will be most accurate if they include both indicator types. However, the hypothesis that improved soil health boosts

crop productivity was not supported. While improved soil biological, chemical, and physical properties may directly support higher crop yields in systems where water or nutrients are limiting, they are less likely to do so in the irrigated and fertilized systems which are common in California. Including factors like potential input reduction, soilborne disease incidence, or yield stability over time may allow for a more accurate assessment of the potential for a particular soil health building practice to promote crop performance.

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Appendix 1: Supplementary Information for Chapter 1

Supplemental methods

For the main body of the experiment, we also modeled N mineralization potential and rate constants for all amendments in each incubation. Potential net N mineralization of the added organic N (N_0) and rate constant (k) were calculated for each amendment at time t using first order kinetics as

$$N_{or} = N_0 * (1 - e^{-kt})$$

(Stanford and Smith 1972), using PROC NLIN in SAS 9.4 (SAS Institute, Cary NC). Mineralization parameters were calculated for each individual replicate within each amendment and soil. Amendments were considered to follow first order kinetics when solutions converged for all replicates. Mineralization parameters were compared using PROC MIXED in SAS such that each incubation date (Spring 2017, Fall 2017, and Fall 2018) was analyzed as a separate randomized complete block design experiment with replicates as blocks. Blocks were treated as random effects while treatment and soil type were fixed effects. One amendment, GF4%, was incubated at each date to ensure conditions were comparable.

Incubation dates were compared by testing the GF4% date and date by soil interactions for N_0 and k, using PROC MIXED as described above. Both parameters were log-transformed to meet the assumption of homogeneity of variance.

Appendix 1 Table 1. Measured mineral N as a percent of the initial added N after 7, 21, 42, and 84 d of incubation at 23 °C and 60% WHC. Different lowercase letters indicate differences among amendments within a soil type and sampling date. GF=Granular fertilizer. PF=pelleted fertilizer. PMC-S,M: poultry manure composts from the Sutter, Merced facilities. YTC=Yard trimmings composts. Feather= feather meal. Fish: fish emulsion. Food and Food (shaken): shaken and unshaken food hydrolysate. Verm=Vermicompost. Blood: Blood meal. GF4% is a granular fertilizer which was included at each amendment date to ensure comparable experimental conditions.

Incubation date	Amd	Nmin7		Nmin21		Nmin42		Nmin84	
		CONV	ORG	CONV	ORG	CONV	ORG	CONV	ORG
Spring 2017	GF4%-1	41.14 *b*	36.87 *b*	42.06 *b*	43.67 *b*	48.91 *b*	56.15 *b*	53.13 *ab*	54.30 *ab*
Spring 2017	Guano	78.68 *a*	68.43 *a*	85.02 *a*	80.14 *a*	87.46 *a*	92.08 *a*	85.19 *a*	87.89 *a*
Spring 2017	PF6%	34.49 *bc*	29.71 *bc*	41.25 *b*	40.36 *b*	49.80 *b*	52.04 *b*	57.38 *ab*	55.31 *ab*
Spring 2017	PF4%	26.00 *c*	21.19 *c*	29.10 *bc*	26.00 *c*	37.41 *bc*	32.47 *c*	36.98 *b*	40.71 *b*
Spring 2017	PMC-S1	19.30 *c*	19.11 *c*	25.15 *c*	25.69 *c*	29.40 *c*	31.13 *c*	33.31 *b*	36.09 *b*
Spring 2017	YTC-Y2	0.90 *d*	0.83 *d*	0.82 *d*	0.54 *d*	0.77 *d*	-0.56 *d*	0.76 *c*	-1.49 *c*
Spring 2017	YTC-Y4	0.84 *de*	0.31 *d*	0.22 *d*	0.14 *d*	-0.35 *de*	-1.33 *d*	-1.48 *c*	-2.23 *c*
Spring 2017	YTC-Y1	0.10 *e*	-0.25 *d*	-1.12 *de*	-0.94 *de*	-1.26 *de*	-2.49 *de*	-2.21 *c*	-3.34 *c*
Spring 2017	YTC-Y3	-1.82 *e*	-3.53 *e*	-3.36 *e*	-3.22 *e*	-2.95 *e*	-3.96 *e*	-3.15 *c*	-1.15 *c*
Fall 2017	GF4%-2	34.76 *a*	31.80 *a*	42.93 *b*	40.07 *b*	45.75 *bc*	44.46 *bc*	52.27 *ab*	48.38 *b*
Fall 2017	Feather	42.81 *a*	40.59 *a*	59.21 *a*	55.71 *a*	64.54 *a*	61.75 *a*	66.75 *ab*	65.75 *ab*
Fall 2017	Fish	41.55 *a*	41.38 *a*	56.98 *ab*	55.96 *a*	60.41 *ab*	65.66 *a*	67.19 *a*	70.56 *a*
Fall 2017	Food	45.39 *a*	45.80 *a*	52.29 *ab*	55.75 *a*	58.99 *ab*	60.76 *a*	65.52 *ab*	66.93 *a*
Fall 2017	GF2%	21.68 *b*	23.98 *b*	30.45 *c*	28.74 *c*	34.76 *c*	35.93 *c*	38.72 *bc*	37.51 *bc*
Fall 2017	PMC-M	17.41 *b*	18.86 *b*	22.95 *c*	25.56 *c*	24.63 *c*	26.16 *d*	27.61 *c*	27.84 *c*
Fall 2017	PMC-S2	17.15 *b*	15.99 *b*	24.63 *c*	28.99 *c*	28.63 *cd*	27.08 *d*	31.08 *c*	29.34 *c*
Fall 2017	Verm	14.67 *b*	17.18 *b*	15.85 *d*	15.47 *d*	15.07 *d*	14.54 *e*	16.25 *d*	14.01 *d*
Fall 2017	YTC-S	2.00 *c*	3.47 *c*	3.14 *e*	2.39 *e*	3.87 *e*	3.84 *f*	4.98 *e*	3.09 *e*
Fall 2018	GF4%-3	33.68 *b*	38.70 *b*	42.20 *b*	43.04 *b*	43.41 *b*	51.52 *b*	49.99 *b*	55.30 *b*
Fall 2018	Food2	46.18 *a*	50.07 *a*	63.03 *a*	64.64 *a*	60.38 *a*	66.88 *a*	65.02 *a*	75.33 *a*
Fall 2018	Blood	44.74 *a*	38.89 *ab*	58.41 *ab*	59.82 *a*	68.57 *a*	66.78 *a*	71.64 *a*	70.33 *ab*
Fall 2018	PMC-S3	13.33 *c*	13.23 *c*	18.72 *c*	15.97 *c*	22.49 *c*	22.05 *c*	30.04 *c*	28.66 *c*
Fall 2018	YTC/PMC	11.32 *c*	10.28 *c*	11.61 *d*	12.31 *c*	12.56 *d*	15.83 *d*	14.27 *d*	18.47 *d*
Fall 2018	YTC-Y5	2.04 *d*	2.05 *d*	2.74 *e*	1.87 *e*	4.15 *e*	3.67 *e*	2.92 *e*	2.71 *e*

Appendix 1 Table 2 Modeled amendment N mineralization rate constants (k) and potential net N mineralization (N0) for organic amendments (amd) incorporated into soils from conventionally (CONV) and organically (ORG) managed fields incubated in Spring and Fall 2017 and Fall 2018, and significance of the effect of soil, amendment, and their interactions. Lowercase letters represent differences among amendments within a soil type and date. PMC-S: poultry manure composts from the Sutter facility. PF: pelleted fertilizer. Fish: fish emulsion. Food and Food (shaken): shaken and unshaken food hydrolysate. GF: granular fertilizer. GF4% is a granular fertilizer which was included at each amendment date to ensure comparable experimental conditions.

Incubation date	Amendment ID	k (day^{-1})				N_0 (% of organic N mineralized)				Effect	k	N_0
		CONV		ORG		CONV		ORG			p-value	
Spring 2017	*GF4%*	*0.153*	*a*	*0.067*	*a*	*28.72*	*ab*	*33.74*	*ab*	*Amend*	0.0010	<.0001
	PMC-S1	0.030	*b*	0.026	*a*	18.69	*b*	22.88	*b*	*Soil*	0.0054	0.0043
	PF4%	0.105	*ab*	0.041	*a*	26.68	*b*	31.15	*ab*	*Amend*soil*	0.7644	0.5264
	PF6%	0.114	*ab*	0.084	*a*	48.01	*a*	50.09	*a*			
	Guano	0.187	*a*	0.076	*a*	27.91	*b*	38.10	*ab*			
Fall 2017	*GF4%*	*0.077*	*a*	*0.067*	*a*	*27.12*	*b*	*27.82*	*b*	*Amend*	0.0004	<.0001
	GF2%	0.044	*a*	0.042	*a*	23.23	*b*	20.51	*b*	*Soil*	0.964	0.9658
	Feather	0.146	*a*	0.134	*a*	63.87	*a*	62.12	*a*	*Amend*soil*	0.8765	0.6927
	Fish	0.102	*a*	0.084	*a*	48.39	*a*	54.80	*a*			
	Food	0.143	*a*	0.141	*a*	47.72	*a*	49.73	*a*			
Fall 2018	*GF4%*	*0.055*	*b*	*0.050*	*b*	*23.23*	*b*	*26.39*	*b*	*Amend*	<.0001	<.0001
	Blood	0.136	*a*	0.113	*a*	67.76	*a*	67.84	*a*	*Soil*	0.1612	0.7633
	Food (shaken)	0.161	*a*	0.144	*a*	51.61	*a*	58.67	*a*	*Amend*soil*	0.5837	0.6569

Appendix 1 References

Stanford, G., and S.J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. J. 36:465–472. doi:10.2136/sssaj1972.03615995003600030029x.

Appendix 2 Table 1 Summary of carbon (C) and nitrogen (N) assays and measured and calculated variables. Incubations performed in sealed jars (2 vials/jar) at 25°C and 60% water holding capacity. Ammonium (NH_4-N), nitrate (NO_3-N) and total organic C (TOC) analyses were performed on 1:5 soil:K_2SO_4 extracts, and CO_2-C analysis on headspace air sampled from each jar via a rubber septum.

Vial	Treatment	Analyses
A	Immediate extraction with 0.5 M K_2SO_4	NH_4-N, NO_3-N, total organic C (TOC)
B	Immediate fumigation with chloroform, extraction	TOC
C	7 d incubation with *V. faba* residue, extraction	CO_2-C, NH_4-N, NO_3-N, TOC
D	7 d incubation with *V. faba* residue, fumigation, extraction	CO_2-C, TOC
E	7 d incubation (no residue), extraction	CO_2-C, NH_4-N, NO_3-N, TOC
F	7 d incubation (no residue), extraction, fumigation	CO_2-C, TOC

Category	Variable	Description	Measurements	Calculated as
C cycle	MBC_0	Microbial biomass C at sampling	TOC	B-A
	MBC-Res	Apparent MBC growth when incubated with residues for 7 d	TOC	(D-C)-(F-E)
	CO_2-Soil	Respiration from SOM over 7 d	CO_2-C	Jar (EF)
	CO_2-Res	Additional respiration from ground residues	CO_2-C	Jar (CD)-Jar(EF)
N cycle	$Nmin_0$	Mineral N (NH4-N+ NO3-N) at sampling	NH_4-N + NO_3-N	A
	Nmin-Soil	Additional net N mineralization from SOM over 7 d incubation	NH_4-N + NO_3-N	E-A
	Nmin-Res	Additional net N mineralization from added residues	NH_4-N + NO_3-N	C-E
	NH_4-Res	Proportion of the mineral N measured after 7 d incubation with residues present as NH_4-N	NH_4-N /(NH_4-N+NO_3-N)	C

Appendix 3 Table 1a Mean values of indicators in the CONV sstem (See text for abbreviations).

		Mgt	Conv							
		Crop	Corn				Tomato			
		Date	Spring		Summer		Spring		Summer	
		Year	2018	2019	2018	2019	2018	2019	2018	2019
Chemical indicato	**EC**	µS cm^{-1}	105	111	97	101	94.2	88.3	76.3	91.6
	pH		7.60	7.67	7.90	7.79	7.67	7.63	8.03	7.60
	Mineral N	ppm	6.83	7.73	3.05	3.87	4.39	5.73	2.06	5.79
	Olsen P	ppm	32.2	64.7	37.6	42.1	28.4	48.1	22.1	71.7
	Ca	ppm	2243	2027	2270	2107	2082	2090	2211	2181
	Mg	ppm	1929	1848	1970	1906	1808	1945	1899	2072
	K	ppm	186	206	149	155	167	230	132	175
	Na	ppm	44.0	51.1	59.0	56.2	37.9	41.4	56.8	31.4
Organic matter	**Total N**	%	0.10	0.10	0.10	0.09	0.10	0.10	0.09	0.10
pool indicators	**Total C**	%	0.97	0.96	0.94	0.89	0.88	0.97	0.84	0.93
	Tot. C:N		9.41	9.65	9.54	9.54	8.82	9.71	9.24	9.69
	MBC	ppm	160	201	147	119	170	191	121	211
	POXC	ppm	299	347	281	327	301	338	256	315
	POM-N	ppm	74.1	115	97.9	98.2	86.1	81.1	96.9	111
	POM-C	ppm	777	1201	1015	946	891	852	904	1142
	POM C:N		10.6	10.4	10.5	9.76	10.8	10.4	9.7	10.3
Bio. indicators	**FDA**	ppm hr^{-1}	7.98	13.0	7.66	8.89	8.24	11.79	6.91	8.75
	Rewet CO_2-C	ppm 3d^{-1}	36.9	66.1	35.2	49.7	39.7	56.8	35.8	54.6
Phys. indicators	**Aggregates**		0.45	0.34	0.46	0.27	0.30	0.40	0.37	0.36
	Bulk D.	g cm^{-3}	1.29	1.35	1.29	1.35	1.34	1.33	1.34	1.33

Appendix 3 Table 1b Mean values of indicators in the ORG system

		Mgt	Org							
		Crop	Corn				Tomato			
		Date	Spring		Summer		Spring		Summer	
		Year	2018	2019	2018	2019	2018	2019	2018	2019
Chem. indicators	**EC**	µS cm^{-1}	219	242	201	205	186	223	146	198
	pH		7.11	7.30	7.66	7.35	7.36	7.33	7.78	7.23
	Mineral N	ppm	22.1	25.3	5.5	29.5	12.8	23.3	6.9	23.7
	Olsen P	ppm	43.2	70.1	38.3	77.1	39.3	62.9	32.2	78.9
	Ca	ppm	2607	2386	2556	2506	2638	2346	2649	2383
	Mg	ppm	1800	1692	1724	1797	1792	1695	1800	1747
	K	ppm	336	392	226	302	266	371	198	285
	Na	ppm	75.9	86.2	92.3	85.8	78.1	111.3	90.1	79.8
Organic matter pool indicators	**Total N**	%	0.17	0.17	0.14	0.15	0.17	0.16	0.14	0.15
	Total C	%	1.37	1.42	1.24	1.28	1.38	1.35	1.22	1.22
	Tot. C:N		8.25	8.55	8.65	8.49	8.31	8.67	8.62	8.41
	MBC	ppm	385	455	358	260	410	417	274	365
	POXC	ppm	477	521	393	450	506	489	399	410
	POM N	ppm	191	202	173	188	187	182	147	200
	POM C	ppm	1985	2176	1684	1846	2103	1876	1462	1826
	POM C:N		10.5	10.8	9.8	9.84	11.3	10.3	9.9	9.2
Bio. indicators	**FDA**	ppm hr^{-1}	20.2	35.2	16.9	25.7	20.7	26.7	14.4	18.1
	Rewet CO_2-C	ppm 3d^{-1}	78.0	126.0	58.6	95.3	79.4	124.9	62.7	85.0
Phys. indicators	**Aggregates**		0.47	0.46	0.56	0.40	0.44	0.43	0.60	0.42
	Bulk D.	g cm^{-3}	1.20	1.41	1.20	1.41	1.30	1.34	1.30	1.34

Appendix 3 Table 2 P-values for all main effects and interactions. Bolded values are significant at p<0.05

				Chemical indicators				
Effect	EC	pH	Min. N	Olsen P	Ca	Mg	K	Na
Mgt	**<.0001**	**<.0001**	**<.0001**	**0.0329**	**0.0006**	**0.0247**	**<.0001**	**<.0001**
Crop	**0.0001**	0.2267	**0.0006**	0.6002	0.5403	0.6699	0.1831	**0.0163**
Date	**0.0024**	**<.0001**	**<.0001**	0.7654	**0.0078**	**0.0071**	**<.0001**	0.0685
Year	**0.0025**	**0.0004**	**<.0001**	**<.0001**	**0.0004**	0.8947	**0.0002**	0.6017
Mgt*Crop	0.5179	0.2004	0.8025	0.8255	0.8198	0.7743	0.1723	**0.005**
Mgt*Date	0.3603	0.3978	0.4864	0.7687	0.1415	0.1158	0.1347	0.1388
Mgt*Year	0.0694	0.3674	**<.0001**	0.4739	0.1316	0.102	0.2387	0.0651
Crop*Date	0.5982	0.0989	0.0628	0.2744	0.5307	0.261	0.5075	0.0692
Crop*Year	0.2588	**0.0069**	0.1446	0.3784	0.7883	0.4839	0.223	0.1617
Year*Date	0.3854	**<.0001**	**0.0003**	0.3284	0.2123	**0.0488**	0.4211	**0.0001**
Mgt*Crop*Date	0.6686	0.4036	0.9364	0.4604	0.351	0.5544	0.5192	0.7932
Mgt*Crop*Year	0.326	0.9861	0.1348	0.5042	0.1698	0.2464	0.731	0.0581
Mgt*Year*Date	0.5846	**0.0485**	0.0725	0.3775	0.2804	0.2	**0.0442**	0.4169
Crop*Year*Date	0.1336	0.3174	0.6565	0.0768	0.1507	0.421	0.3665	0.0332

				Organic matter indicators				
	Total N	Total C	Total C:N	MBC	POXC	POM N	POM C	POM C:N
Mgt	**<.0001**	**0.0002**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	0.8889
Crop	0.0508	**0.0341**	0.257	0.0914	0.0578	0.3027	0.115	0.7355
Date	**<.0001**	**<.0001**	0.063	**<.0001**	**<.0001**	0.401	0.1662	**0.0014**
Year	0.9699	0.0966	**0.0065**	**0.0077**	**<.0001**	**0.0222**	**0.004**	0.3932
Mgt*Crop	0.8697	0.5881	0.1731	0.2127	0.4958	0.8038	0.7369	0.9653
Mgt*Date	**0.0037**	**0.0003**	0.9257	0.1719	**0.0002**	**0.0023**	**0.0018**	0.1565
Mgt*Year	0.3824	0.5058	**0.0408**	0.0575	**0.0037**	0.76	0.3413	0.5809
Crop*Date	0.7389	0.7213	0.8685	**0.0376**	0.0516	0.1737	0.4688	0.5047
Crop*Year	0.7592	0.5683	0.1742	**0.0375**	0.3928	0.7109	0.3324	0.9154
Year*Date	**0.0092**	0.9159	**0.0007**	0.4266	**0.0255**	0.6433	0.7379	0.993
Mgt*Crop*Date	0.3549	0.75	0.1323	0.2202	0.6369	0.294	0.2712	0.6183
Mgt*Crop*Year	0.3893	0.2927	0.1629	0.5951	0.2696	0.382	0.6757	0.3521
Mgt*Year*Date	0.1069	0.2642	0.3483	0.2776	0.8198	**0.0175**	0.0525	0.864
Crop*Year*Date	0.0757	0.1645	0.47	**<.0001**	0.3054	**0.0007**	**0.001**	0.2832

	Biological processes			*Physical indicators*	
	FDA	Cmin 72		Agg. Stab.	Bulk dens.
Mgt	**<.0001**	**<.0001**		**0.0217**	0.426
Crop	**0.0332**	0.7745		0.2555	0.4692
Date	**<.0001**	**<.0001**		0.1805	
Year	**<.0001**	**<.0001**		**<.0001**	**0.0026**
Mgt*Crop	0.1811	0.8612		0.1961	0.9943
Mgt*Date	0.1062	**0.0011**		**0.0491**	
Mgt*Year	0.524	**0.0028**		0.0511	**0.0309**
Crop*Date	0.0928	0.8357		0.0646	
Crop*Year	0.2648	0.2634		0.2364	**0.0089**
Year*Date	**0.0073**	**0.0402**		**0.0002**	
Mgt*Crop*Date	0.3721	0.3512		0.8971	
Mgt*Crop*Year	0.3191	0.7045		0.1771	0.2539
Mgt*Year*Date	0.118	0.3377		0.1753	
Crop*Year*Date	0.1222	0.8792		0.5554	

Appendix 3 Table 3 Descriptive statistics for in-plot CVs, calculated using three adjacent locations within a single bed in each plot for each block*management*crop*date*year combination (n=144).

	In-plot CV (%)				
Indicator†	**Median**	**Mean**	**Min**	**Max**	**St. dev.**
		Chemical indicators			
EC	10	13	2.4	37	8.3
pH	1.1	1.1	0.1	3.0	0.7
Mineral N	17	20	3.3	55	13
Olsen P	16	20	1.8	77	17
Calcium	2.9	3.5	0.7	13	2.2
Magnesium	3.1	3.6	0.6	17	2.8
Potassium	7.0	8.7	0.5	35	6.6
Sodium	11	14	2.2	52	11
		Organic matter pool indicators			
Total N	2.9	3.7	0.4	29	4.2
Total C	3.0	3.7	0.3	15	2.6
Total C:N	1.3	1.8	0.1	12	2.3
MBC	12	13	0.4	34	7.5
POXC	5.4	5.7	0.4	13	2.9
POM-N	11	13	0.6	45	8.2
POM-C	13	16	2.4	39	10
POM C:N	5.0	7.4	1.5	29	5.8
		Biological process indicators			
FDA	12	14	4.4	34	6.6
Rewet CO_2	15	18	1.7	39	10
		Physical indicators			
Aggregate stability	13	14	2.2	38	8.1
Bulk Density	4.3	5.2	0.6	16	3.7

Appendix 4: Supplementary information for Chapter 4

Appendix 4 Table 1 Pearson correlation coefficients between soil health indicators and N mineralization, C storage and yields across the whole dataset (n=24).

Indicator	Carbon stocks		Nitrogen mineralization		Yields (normalized)	
			Chemical indicators			
EC	0.87	***	0.78	***	-0.77	***
pH	-0.84	***	-0.52	**	0.78	***
Mineral N	0.79	***	0.82	***	-0.67	***
Olsen P	0.39		0.77	***	-0.13	
Ca	0.84	***	0.25		-0.87	***
Mg	-0.15		-0.47	*	0.17	
K	0.83	***	0.83	***	-0.62	**
Na	0.84	***	0.71	***	-0.67	***
			Organic matter pool indicators			
C stocks	--		0.65	***	-0.86	***
Total N	0.96	***	0.64	***	-0.89	***
Total C:N	-0.56	**	-0.34		0.65	***
MBC	0.94	***	0.77	***	-0.82	***
POM N	0.82	***	0.70	***	-0.80	***
POM C	0.88	***	0.69	***	-0.84	***
POM C:N	0.27		-0.04		-0.21	
POXC	0.95	***	0.74	***	-0.84	***
			Biological process indicators			
Nmin-28d	0.65	***	--		-0.44	*
FDA	0.81	***	0.92	***	-0.66	***
Cmin-1d	0.82	***	0.88	***	-0.64	***
Cmin-3d	0.79	***	0.95	***	-0.56	**
Cmin-28d	0.91	***	0.80	***	-0.73	***
			Physical indicators			
Moisture	0.78	***	0.55	***	-0.64	***
Aggregates	0.60	**	0.20		-0.59	**
Sand	-0.35		-0.02		0.26	
Silt	0.40		0.06		-0.30	
Clay	0.22		-0.06		-0.13	
			Plant indicators (normalized)			
Early Leaf N	-0.29		-0.72	***	0.09	
Mid. Leaf N	-0.30		-0.27		0.37	
Early CF	0.30		0.36		-0.01	
Mid. CF	0.31		0.44	*	-0.24	
Yields	-0.86	***	-0.44	*	--	

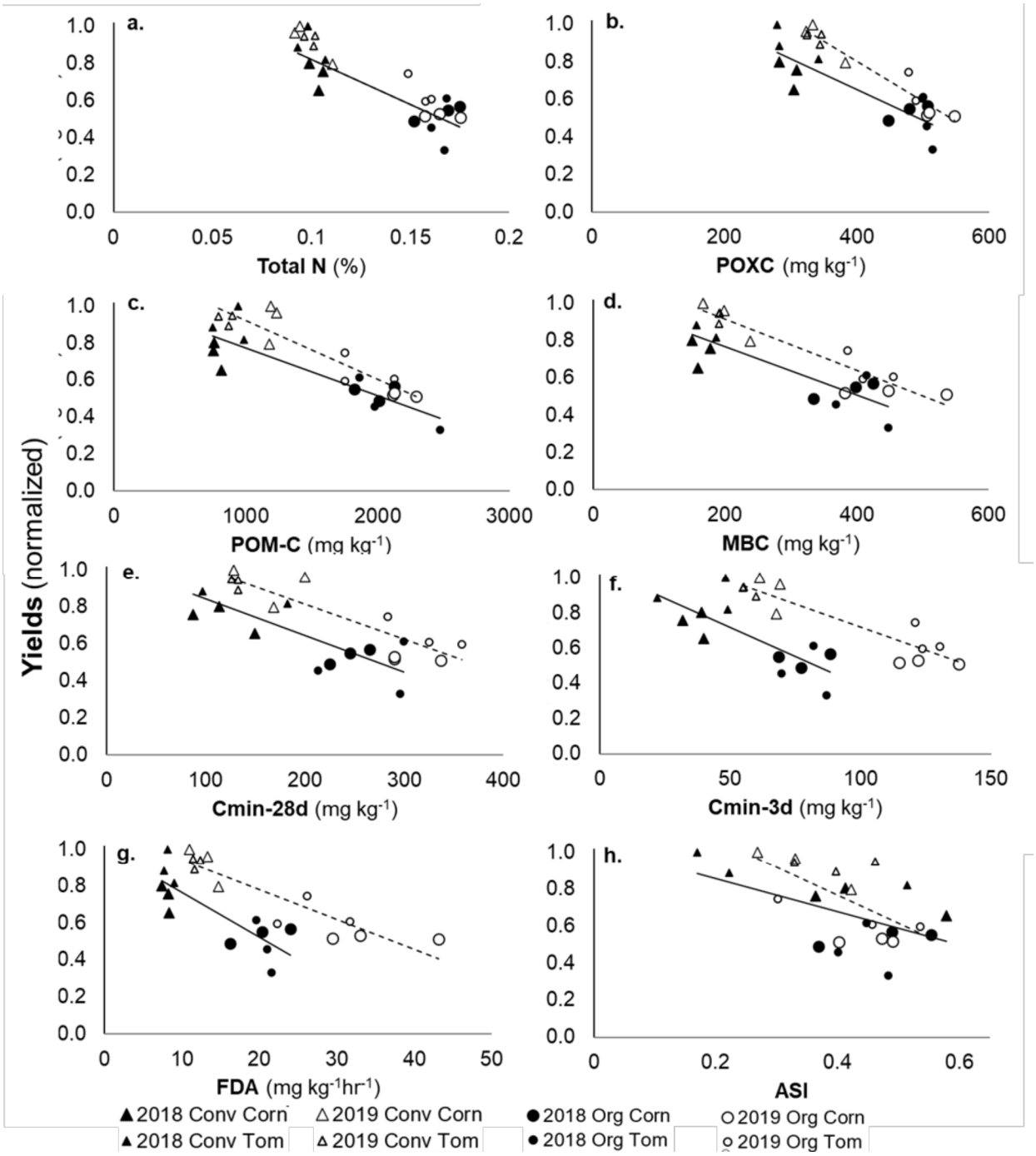
†EC, electrical conductivity; MBC, microbial biomass C; POXC, permanganate oxidizable C; POM, particulate organic matter; Nmin-28d, net N mineralization from rewet soil over 28d aerobic incubation; FDA, fluorescein diacetate hydrolysis; Cmin-1d,3d,28d, CO_2-C measured from rewet soil after 1,2, and 28 d of aerobic incubation; ASI, aggregate stability index; Bulk D., bulk density; Early and Mid leaf N, N concentraiton in a recently matured leaf during early and midseason growth; Early and Mid CF, chlorophyll fluorescence in a recently matured leaf during early and midseason growth

* Significant at p<0.05 **Significant at p<0.01 ***Significant at p<0.001

Appendix 4 Table 2 Average mean values for soil indicators measured on conventional (CONV) and organic (ORG) plots prior to planting corn and tomato in 2018 and 2019, and plant indicators measured for corn and tomato crops in 2018 and 2019 (n=3).

	Crop		Corn				Tomato			
	Year		2018		2019		2018		2019	
	Mgt		CONV	ORG	CONV	ORG	CONV	ORG	CONV	ORG
Chem. indicators	**EC**[†]	(µS cm^{-1})	105	219	111	242	94	186	88	223
	pH		7.60	7.11	7.67	7.30	7.67	7.36	7.63	7.33
	Mineral N	(ppm)	6.83	22.08	7.73	25.32	4.4	12.8	5.7	23.3
	Olsen P	(ppm)	32.2	43.2	64.7	70.1	28.4	39.3	48.1	62.9
	Ca	(ppm)	2243	2607	2027	2386	2082	2638	2090	2346
	Mg	(ppm)	1929	1800	1848	1692	1808	1792	1945	1695
	K	(ppm)	186	336	206	392	167	266	230	371
	Na	(ppm)	44.0	75.9	51.1	86.2	37.9	78.1	41.4	111.3
Organic matter pool indicators	**C stocks**	(Mg ha^{-1})	19.1	26.4	18.9	27.3	17.8	27.9	19.6	27.4
	Total N	(%)	0.10	0.17	0.10	0.17	0.10	0.17	0.10	0.16
	Tot. C:N		9.41	8.25	9.65	8.55	8.82	8.31	9.71	8.67
	MBC	(ppm)	160	385	201	455	170	410	191	417
	POXC	(ppm)	299	477	347	521	301	506	338	489
	POM N	(ppm)	74.1	191	115	202	86	187	81	182
	POM C	(ppm)	777	1985	1201	2176	891	2103	852	1876
	POM C:N		10.6	10.5	10.4	10.76	10.8	11.3	10.4	10.3
Bio. indicators	**Nmin-28d**	(ppm)	11.7	24.2	26.2	60.4	13.1	25.8	21.3	57.5
	FDA	(ppm hr^{-1})	7.98	20.18	13.0	35.19	8.2	20.7	11.8	26.7
	Cmin-1d	(ppm)	21.3	43.9	32.6	61.4	23.3	43.6	26.6	60.3
	Cmin-3d	(ppm)	36.9	78.0	66.1	126	39.7	79.4	56.8	125
	Cmin-28d	(ppm)	117	245	165	305	136	269	130	322
Phys. indicators	**ASI**		0.45	0.47	0.34	0.46	0.30	0.44	0.40	0.43
	Bulk D.	(g cm^{-3})	1.29	1.20	1.35	1.41	1.34	1.30	1.33	1.34
	Sand	(%)	19.8	23.9	25.7	20.9	25.7	20.9	19.8	23.9
	Silt	(%)	61.0	58.2	56.3	60.5	56.3	60.5	61.0	58.2
	Clay	(%)	19.2	17.9	18.0	18.6	18.0	18.6	19.2	17.9
Plant indicators	**Early leaf N**	(%)	4.55	4.57	4.42	3.65	5.85	5.47	4.89	4.89
	Mid. leaf N	(%)	2.91	2.47	2.36	2.19	4.31	4.24	4.96	4.79
	Early CF		0.76	0.79	0.80	0.80	0.85	0.84	0.85	0.85
	Mid CF		0.80	0.83	0.80	0.82	0.81	0.82	0.86	0.87
	Yields	(Mg ha^{-1})	13.9	10.1	17.4	9.8	98.8	51.3	102	71.2

[†]EC, electrical conductivity; MBC, microbial biomass C; POXC, permanganate oxidizable C; POM, particulate organic matter; Nmin-28d, net N mineralization from rew et soil over 28 d aerobic incubation; FDA, fluorescein diacetate hydrolysis; Cmin-1d,3d,28d, CO_2-C measured from rew et soil after 1, 3, and 28 d of aerobic incubation; ASI, aggregate stability index; Bulk D., bulk density; Early and Mid leaf N, N concentration in a recently matured leaf during early and midseason grow th; Early and Mid CF, chlorophyll fluorescence in a recently matured leaf during early and midseason grow th



Appendix 4 Figure 1. Relationship between crop yields (normalized to the highest value for the respective crops) and a) total soil N; b) permanganate oxidizable C (POXC); c) Particulate organic matter C (POM-C); d) Microbial biomass C (MBC); rewet CO_2-C respired after incubation at 22 °C and 60% water-holding capacity for e) 28 d (Cmin-28d) and f) 3 d (Cmin-3d); g) fluorescein diacetate hydrolysis rate (FDA); and h) aggregate stability index (ASI). Conv=Conventional, Org=Organic, Corn= corn was planted in the sampling year and Tom=tomato was planted in the sampling year. Solid lines represent the linear relationship for 2018 and dotted lines the linear relationship for 2019.