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NITROGEN MINERALIZATION POTENTIAL AS INFLUENCED BY MICROBIAL BIOMASS, COTTON RESIDUES AND TEMPERATURE

Bruce A. Roberts,[1] Felix B. Fritschi,[2] William R. Horwath,[3] and Sougata Bardhan[4]

[1]*Department of Plant Science, University of California, Fresno, California, USA*
[2]*Division of Plant Sciences, University of Missouri, Columbia, Missouri, USA*
[3]*Land, Air and Water Resources, University of California, Davis, California, USA*
[4]*Center for Agroforestry, University of Missouri, Columbia, Missouri, USA*

□ *Integrating information on nitrogen (N) mineralization potentials into a fertilization plan could lead to improved N use efficiency. A controlled incubation mineralization study examined microbial biomass dynamics and N mineralization rates for two soils receiving 56 and 168 kg N ha^{-1} in a Panoche clay loam (Typic Haplocambid) and a Wasco sandy loam (Typic Torriorthent), incubated with and without cotton* (Gossypium hirsutum *L.) residues at 10 and 25° C for 203 days. Microbial biomass activity determined from mineralized carbon dioxide (CO_2) was higher in the sandy loam than in clay loam independent of incubation temperature, cotton residue addition and N treatment. In the absence of added cotton residue, N mineralization rates were higher in the sandy loam. Residue additions increased N immobilization in both soils, but were greater in clay loam. Microbial biomass and mineralization were significantly affected by soil type, residue addition and temperature but not by N level.*

Keywords: crop residue, nitrogen mineralization potential, microbial biomass, soil texture, N fertility

INTRODUCTION

Nitrogen (N) is the most common limiting nutrient in California cotton (*Gossypium hirsutum* L.) production (Hutmacher et al., 2004). Although an estimated 80–90% of the total soil N is in organic form, most of it is unavailable to the plants (Stevenson, 1994; Paul and Clark, 1996). Since most of the N assimilated by plants is derived from inorganic-N sources, mineralization plays an important role in controlling the amounts of N available to

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Address correspondence to Bruce A. Roberts, Department of Plant Science, University of California, 2415 E. San Ramon Ave., M/S AS72, Fresno, CA 93740-8033, USA. E-mail: baroberts@csufresno.edu

plants (Barrios et al., 1996; Okonkwo et al., 2008). Soil N status can change with addition of carbon source and it is often extremely difficult to predict the actual N needs for cotton. Although, decomposing crop residues can enhance N availability in soils (Egelkraut et al., 2000; Fritschi et al., 2005), currently, N recommendations for California cotton are based on estimated amounts of residual inorganic N at the beginning of the cropping season. However, such evaluations fail to consider the N mineralization potential of a particular field (Weir et al., 1996). Excessive application of N fertilizer for crop production has led to non-point source pollution mainly through transport of nitrate (NO_3^-) in surface and ground waters (Ribbe et al., 2008; Kite-Powell and Harding, 2006; Mengel, 1991). In addition to problems with non-point pollution, over-application of N in cotton production can also result in reductions in yield (Bouqe and Moore, 2000).

In order to improve N use efficiency (NUE, i.e., greater dry matter produced per unit N applied) and prevent NO_3^- pollution it is necessary to adjust N fertilization rates according to the N mineralization potential of a soil (Mulvaney et al., 2005; Franco and Cady, 1997; Mengel, 1991). Nitrogen mineralization refers to the conversion of organic nitrogen to plant available inorganic forms. Microbial biomass, although a small part of the total soil organic matter, plays a critical role in soil nutrient cycling (Bilen et al., 2011; Bolton et al., 1985; Horwath and Paul, 1994; Smith and Paul, 1990). Decomposition of organic matter and subsequent release of inorganic N in soil is carried out mainly by soil bacteria and fungi (Barea et al., 2005). Nitrogen cycling by microbial processes has been shown to be influenced by pH, soil type and management practices (Doran, 1987; Schnurer et al., 1985; Egelkraut et al., 2000; Colloff et al., 2008; Nicol et al., 2008; Hayden et al., 2010; Wakelin et al., 2011). In order to understand more completely the nutrient dynamics of a soil, it is helpful to examine the impact of microbial biomass activity on nutrient availability (Wang et al., 2001).

The objective of this study was to examine the effects of soil type, residue addition, and temperature on microbial biomass carbon (C), microbial biomass N and the kinetics of N mineralization on two of California's major soil types. Incorporating basic processes of nutrient cycling would facilitate soil specific N fertilization recommendations that include the dynamic nature of N mineralization and N mineralization potentials of a particular soil. This would minimize negative environmental impacts and improve NUE and profitability for growers.

MATERIALS AND METHODS

Post-harvest soil samples were taken from a Panoche clay loam (Typic Haplocambid) and a Wasco sandy loam (Typic Torriorthent) as described in Fritschi et al. (2003) to determine soil microbial biomass by the

TABLE 1 Soil characteristics of the two study sites (after Fritschi et al., 2003)

				%			
Location	Soil type	pH	CEC	OM	Clay	Sand	Silt
Panoche cl	Typic Haplocambid	7.8	27	0.66	31	25	44
Wasco sl	Typic Torriorthent	6.8	13	0.78	7	66	27

fumigation-incubation method (Horwath and Paul, 1994). Samples were collected out of 56 and 168 kg N ha^{-1} treatments from two N rate field trials for cotton (Fritschi et al., 2003; Hutmacher et al., 2004). A comparison of soil physical and selected chemical properties of these soils is presented in Table 1. Triplicate soil samples were collected from the planting bed of four replications of each N treatment and combined, thoroughly mixed, prescreened through a 5 mm sieve and stored at 4°C until initiation of the incubation study (Jenkinson and Powlson, 1980).

Incubation Setup

Upon removal from the cold room, soil samples were equilibrated to 55% water holding capacity, corresponding to 20 g and 30 g of water per 100 g of dry soil for the Wasco sandy loam and the Panoche clay loam, respectively (moisture calculations were based on gravimetric water contents as determined by drying samples at 105°C for 24 hrs). Equilibrated soil samples were passed through a 5 mm screen and the equivalent of 100 g dry soil was weighed into 120 mL polypropylene specimen containers (ThermoFisher Scientific Inc., Waltham, MA, USA). Half of the samples received 0.4 g of dry field collected cotton residues (cultivar 'Acala Maxa') that included stems, leaves and roots which were coarsely ground (2 mm) and added and thoroughly mixed with the soil. To approximate the incorporation of residue with tillage under field conditions, the amount of plant material to be added was calculated to represent the addition of 7,500 kg ha^{-1} of biomass (including root biomass) to the top 0.15 m of the soil. Subsamples of dried cotton residue were ball-milled and analyzed for total C and N (DANR Analytical Lab, UC Davis, Davis, CA, USA). Cotton residues used during this experiment were analyzed by standard procedures as described by Robertson and van Soest (1980). The C/N ratio and chemical makeup of the labeled residue are shown in Table 2. After mixing, each specimen container was placed into a 0.95-L jar containing 2 mL of water to prevent soil desiccation. The jars closed with lids that were fitted with rubber septa to allow repeated head-space air sampling. The prepared soil samples were incubated in the dark and under controlled conditions of either 10 or 25°C for 203 days. All treatments were replicated four times for a total of 576 samples. Four replications of each treatment

TABLE 2 Characteristics of cotton residue (after Fritschi et al., 2005)

		g kg^{-1}					
	C/N	C	N	Hemicellulose	Cellulose	Lignin	Minerals
Cotton residues	40.4	362.4	8.96	272.1	327.1	129.9	107.5

were destructively sampled at 0, 6, 14, 21, 35, 62, 90, 127, and 203 days after incubation initiation. Each incubated soil sample was split to determine NO_3^-, ammonium (NH_4^+), and microbial biomass N and C as described below.

Microbial Mineralization of Carbon

Carbon mineralization was determined by measuring carbon dioxide (CO_2) evolution using an Infrared Gas Analyzer. Headspace CO_2 concentration was determined every two to three days during the first 21 days of the incubation interval. Frequent sampling during this initial period was conducted such that headspace CO_2 concentrations were prevented from exceeding 2% between consecutive samplings. Following this initial active period, CO_2 sampling intervals were lengthened while not allowing CO_2 concentration to exceed the 2% threshold. After each gas sampling the incubation jars were aerated with ambient air. The 10°C samples were allowed to reach room temperature before gas analysis.

Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass C (SMBC) was determined by the chloroform-fumigation-incubation method as described by Horwath and Paul (1994). SMBC was calculated according to Horwath et al. (1996) using the following equation:

$$\text{SMBC} = (\text{Fc} - (\text{P} \times \text{UFc}))/\text{Kc}$$

where: Fc = CO_2 flush from the fumigated sample

UFc = CO_2 produced by the control

Kc = 0.41 = the fraction of biomass C mineralized to CO_2

P = K1 (Fc/UFc) + K2 = proportion of UFc that is to be subtracted from the fumigated flush

K1 = 0.29 and K2 = 0.23 are parameters estimated by Horwath et al. (1996) for California soils.

Soil Microbial Biomass Nitrogen (SMBN) was calculated according to Shen et al. (1984) with the following equation:

$$SMBN = Fn/Kn$$

where: Fn = flush of NH4+ due to fumigation
Kn = 0.68 + proportion of microbial N mineralized to NH_4^+ during the 10-d incubation period.

Mineralized N was determined from potassium chloride (KCl) extractable N measured at each sampling date. Standard analysis of variance (ANOVA) was used to determine significance between soils, residue treatment, and temperature and time effects. Statistical analysis was performed using JMP Version 9.0 (SAS Institute, Cary, NC, USA). Significant differences were determined between factors using Fisher's Least Significant Difference (LSD) test.

RESULTS

The design and methodologies of this experiment were selected to compare the effects of soil type and soil N level on soil microbial activity as related to immobilization and mineralization of plant residue N.

Microbial Respiration

The results of the microbial respiration for the different soil types, residue treatments, N levels, and temperatures are presented in Figure 1. Microbial activity of the amended and unamended treatments, as measured by cumulative CO_2 evolution, was significantly greater ($P = 0.0001$) for the Wasco sandy loam than for the Panoche clay loam at 25°C. The addition of plant residue significantly increased CO_2 evolution from microbial respiration of both soils and both N levels. A temperature effect was evident as microbial respiration was significantly ($P = 0.001$) greater at 25°C than 10°C for both soils. The temperature effect on microbial respiration was more evident in the Panoche soil where values of cumulative CO_2 were significantly less than the Wasco Soil at the lower temperature (Figures 1a and 1c) but not at 25°C (Figures 1b and 1d). The field N treatments of 56 and 168 kg N ha^{-1} had a minimal but significant effect on CO_2 evolution, with higher microbial activity observed in the soil samples collected from the 168 kg ha^{-1} than the 56 kg ha^{-1} treatment for both amended and unamended samples.

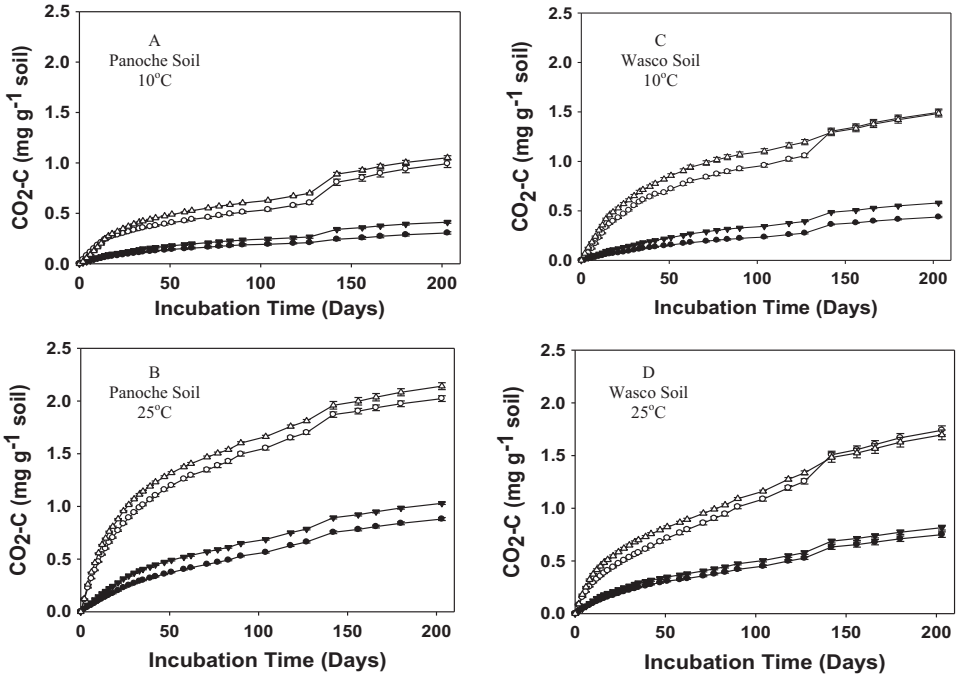

FIGURE 1 Soil respiration for the different treatments in the A, B) Panoche clay loam and the C, D) Wasco sandy loam. •56 kg N ha^{-1}, soil only °56 kg N ha^{-1}, soil plus plant residue, ▼168 kg N ha^{-1}, soil only, Δ168 kg N ha^{-1}, soil plus plant residue.

Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass carbon (MBC) and nitrogen (MBN) are closely associated because of the N requirements for microbial growth. Microbial organisms maintain a fairly constant carbon to nitrogen ratio in soils (Paul and Clark, 1996). Therefore, the levels of the C and N follow similar trends in both soils. Although not significantly different at the $P = 0.05$ level, for the most part, microbial biomass as determined by MBC and MBN started out higher for the Panoche clay loam than for the Wasco sandy loam (Figures 2 and 3). This trend was maintained throughout the incubation. The addition of cotton residues increased the microbial biomass in both soils. There was a general trend in both soils of the incubation at higher temperature resulted in lower final MBC levels. In both soils, the samples from the field treatment receiving higher N applications (168 kg ha^{-1}) ended the incubation period with slightly greater MBC levels than the lower (56 kg ha^{-1}) N treatments.

Net Mineralization

Net mineralization values for the Panoche and Wasco soils as influenced by the addition of plant residue and temperature are shown in Figure 4. N mineralization was significantly greater in the Wasco sandy loam than in the

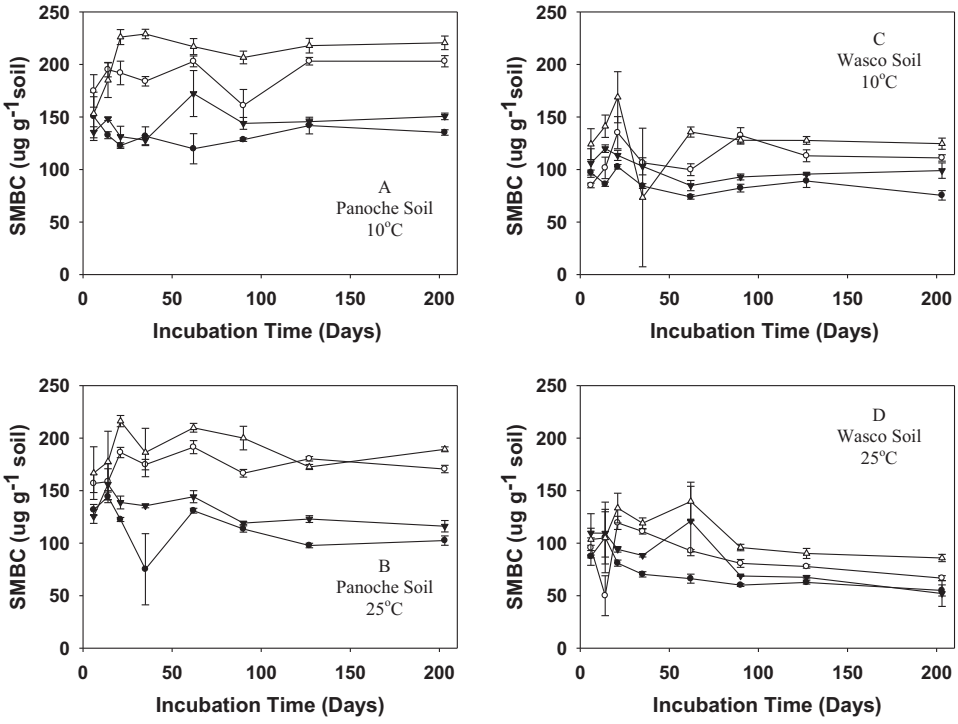

FIGURE 2 Soil microbial biomass carbon (SMBC) for the different treatments in the A, B) Panoche clay loam and the C, D) Wasco sandy loam. •56 kg N ha^{-1}, soil only °56 kg N ha^{-1}, soil plus plant residue, ▼ 168 kg N ha^{-1}, soil only, Δ168 kg N ha^{-1}, soil plus plant residue.

Panoche clay loam. From initiation, N mineralization was significantly higher in the Wasco soil and remained consistently higher throughout the incubation period at both temperatures. Final net mineralization for the Wasco soil was 3.75 times higher than for the Panoche soil. The addition of cotton residue significantly reduced the net mineralization in both soils, but the reduction was greater in the Panoche soil. The suppression of mineralization and the duration of immobilization of the added residues in the Panoche soil resulted in significantly lower final N values over the course of the incubation study. This effect was evident in both the 10 and 25°C treatments. Nitrogen immobilization as a result of added residues in the Panoche soil at 25°C was evident for nearly 100 days of the incubation period. The Wasco soil was less affected by temperature as shown by the increased mineralization of added residue at both temperatures (Figures 4c and 4d). The amount of N mineralized was significantly greater for the 168 kg ha^{-1} treatments than the 56 kg ha^{-1} treatments for both soils ($P = 0.001$). The cumulative N mineralization mean for temperature and added residues were also significantly different ($P = 0.001$). Interactions of N rate by temperature and residue treatments were not significant ($P = 0.72$ and 0.31, respectively).

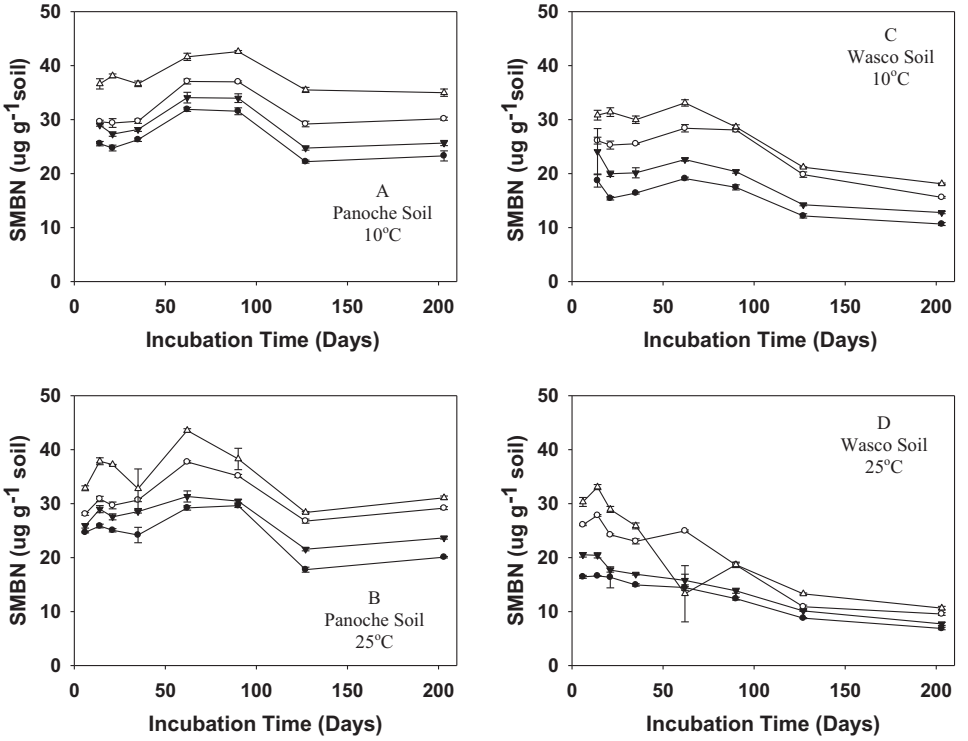

FIGURE 3 Soil microbial biomass nitrogen (SMBN) for the different treatments in A, B) Panoche clay loam and the C, D) Wasco sandy loam (C,D) •56 kg N ha^{-1}, soil only °56 kg N ha^{-1}, soil plus plant residue, ▼ 168 kg N ha^{-1}soil only, Δ168 kg N ha^{-1}, soil plus plant residue.

DISCUSSION

The C and N turnover rates were greater than expected in the Wasco sandy loam than in the Panoche clay loam soil. In fact, the amount of N mineralized in the Wasco soil was two to four times the amount mineralized in the Panoche soil (Figure 4). Further, soil type significantly affected the decomposition rates of added cotton residues and increased temperature raised mineralization rates in both soils albeit not to the same extent. These results were not surprising as N mineralization differences associated with soil texture have been extensively documented, and greater N turnover rates have been observed in sandy loam than in clay loam soil (Kooijman et al., 2009; Matus et al., 2008; Bechtold and Naiman, 2006; Egelkraut et al., 2000; Gordillo and Cabrera, 1997; Ke et al., 1990). Other than physical absorption little has been offered as to why these differences occur. Also, from a management perspective, these results suggest greater contributions of mineralized N to soil N availability in the Wasco soil under field conditions, which in turn may result in differences in crop N uptake between the two soils.

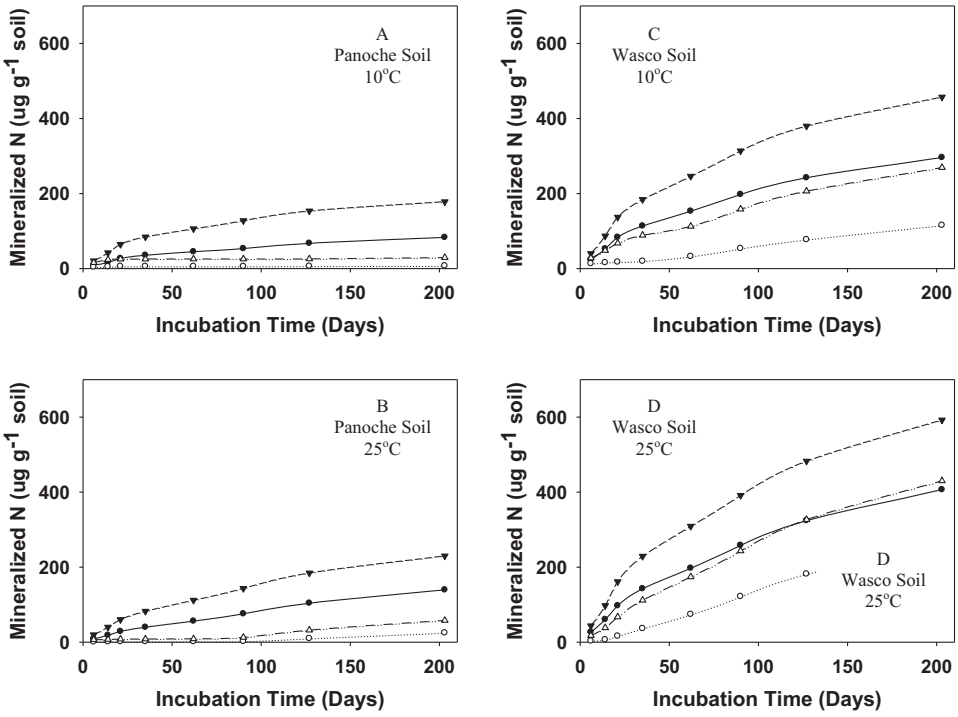

FIGURE 4 Accumulative nitrogen mineralized from A, B) Panoche clay loam and C, D) a Wasco sandy loam. •56 kg N ha^{-1}, soil only °56 kg N ha^{-1}, soil plus plant residue, ▼ 168 kg N ha^{-1}, soil only, Δ168 kg N ha^{-1}, soil plus plant residue.

The addition of cotton residue reduced the cumulative amount of N mineralized over the course of the 203-day incubation more for the Panoche clay loam than the Wasco sandy loam soil. The longer residence time would result in lower turnover rates for cotton residues and lower total mineralization in the clay loam soil compared to the sandy loam soil. This may be due in part to the protective effect of finer soil particles slowing organic residue decomposition as suggested by Egelkraut et al. (2000), Matus et al. (2008), and Mengel (1991). Soil texture effects have been reported as a major factor in N mineralization rates (Stanford and Smith, 1972; Ke et al., 1990; Mengel, 1991; Olness et al., 2001; Kooijman et al., 2009). The accepted explanation is that the protective influence of the smaller pore space is capable of harboring soil organic matter. The results of this study are consistent with this explanation in that the sandy loam soil had the higher mineralization rates.

In addition to temperature, the availability of inorganic N also appeared to limit mineralization more in the Panoche soil than the Wasco soil. The immobilization of N from added residues was more evident in the Panoche soil, and residue decomposition as indicated by microbial activity that was significantly different between the two soils. This suggests the possibility of different microbial responses to available soil N from either oligotrophic

or eutrophic microbial organisms (McKinley and Vestal, 1992; Cotner and Biddanda, 2002; Jurgens and Sala, 2000). It is well established that microbial populations control the turnover rates of soil organic matter (Stevenson, 1994; Paul and Clark, 1996; McKinley and Vestal, 1992). Therefore, since different microbial organisms make up the microbial populations of soils it is reasonable to expect different responses from different microbial populations (Cotner and Biddanda, 2002; Nicol et al., 2008). While there were differences in total microbial biomass measured between these two soils, the differences in mineralization-immobilization dynamics suggest that different microbial communities populate these two soils (Schutter et al., 2001, Cotner and Biddanda, 2002; McKinley and Vestal, 1992). In fact, phospholipid fatty acid analyses conducted on these soils revealed distinct fungal and bacterial signatures (Roberts et al., 2011).

As expected, significantly higher mineralization rates were observed at 25°C compared to 10°C in both soils, and microbial activity was more responsive to temperature in the Panoche soil than in the Wasco soil. These responses indicate that mineralization rate is not only influenced by differences in the protective pore space but that a biological component is also involved. Different responses of the distinct microbial communities to temperature may underlie the observed N mineralization rates. Under field conditions, microbial responses are further modulated by diurnal and seasonal temperature variations per se and the influence of soil water content on temperature dynamics. Therefore, a clay soil that holds more water in the pore spaces would respond differently to temperature fluctuations than soils with less moisture content, thus impacting microbial population and activity.

Microbial biomass as determined by MBC and MBN was greater in the Panoche clay loam than the Wasco sandy loam. In contrast, head-space CO_2 concentration measurements revealed that microbial respiration was greater in the Wasco sandy loam. However, in both soils MBC and MBN initially increased and then stabilized in response to soil amendment with cotton residue. As expected, cotton residue addition increased microbial respiration in both soils and resulted in N immobilization. Nitrogen immobilization was more pronounced in the Panoche clay loam than in the Wasco sandy loam, and persisted longer at 10°C compared to 25°C incubation temperature. The difference in the mineralization rates of the two soils as affected by soil temperature suggest that microbial activity of the Panoche soil is more affected by temperature than the Wasco soil. This would suggest specific thermal kinetic windows of activity for different soils. This concept has been shown to affect enzymatic activities in plant roots (Burk and Upchurch, 1995; McMichael and Burke, 1994; Mahan et al., 1990). From these results it is reasonable to suspect a thermal window of activity applies to soil microbial populations. In addition to micro-sites, clay soil holds more water in the pore spaces and would respond differently to temperature fluctuations than soils

with less moisture content and thus could influence microbial population and activity.

These results illustrate the complexities associated with attempts to predict N mineralization for a given soil under field conditions, and to an even greater extent, use this information to make N recommendations across a broad range of soils. Clearly, under field conditions where aboveground cotton residues are incorporated into the top soil through tillage practices, microbial dynamics and N mineralization-immobilizaton are considerably more complex than in controlled incubation studies. Residue related factors such as distinct particle sizes of leaf, stem, and root tissues and differences in the composition among these fractions influence mineralization rates. Further, management decisions such as shredding of aboveground biomass after harvest, choice of tillage implement and tillage timing and depth of incorporation influence residue decomposition by altering factors of residue-soil contact, residue surface to volume ratios, soil water content, and soil temperature, thus significantly influencing mineralization rates. Therefore, values obtained in this study represent rates observed under controlled conditions. Actual field results have shown similar crop responses to these two soils. The mineralization differences observed is supported by previously reported lint yield, fertilizer use efficiency, and residue-N recovery data from these two field sites.

Fritschi et al. (2003) showed the Panoche soil with significantly lower mineralization rates, experienced an annual decline in lint yields from low N treatments over a three-year study. The low and high N yields from the Wasco soil were not significantly different over the same time period (Fritschi et al., 2003). And while fertilizer use efficiencies (^{15}N dilution) were similar (Fritschi et al., 2004), plant recovery of labeled cotton residue-N averaged 5.9% for the Wasco sandy loam and 3.1% for the Panoche clay loam in the first year following residue applications and 3.2% and 1.7%, respectively, in the second year after application (Fritschi et al., 2005). The importance of seasonal mineralization of organic matter and cotton residues to the N supply of the crop was evident in these studies. The results of this study show different mineralization capacities between these two soils that were significant even when similar residues (same C/N) were incorporated and similar management practices used for three years. The differences between soil incubations at 10°C suggests a biological factor is involved and that soil microbial differences could contribute to nutrient turnover rates in soils as well as organic matter absorption on fine soil fractions.

Improving N-use efficiency will require an understanding of the processes controlling immobilization and mineralization of applied N and residual soil N. Soil type, management inputs and cultural practices affect these biological and chemical processes (Collins et al., 1997; Paustian et al., 1997; Lupwayi et al., 2004; Bardhan et al., 2013). Defining a soil's mineralization potential will improve site-specific crop nutrient guidelines and

recommendations (Mengel, 1991). Soil specific biological factors could also affect the application rates of animal manures and the use of biosolids for land applications (Buyanovsky and Wagner, 1997). As nutrient management plans become the standard for agronomic crop production, crop advisors will need better information on the mineralization capacity of a soil along with other information to make more efficient and accurate recommendations. The role of soil biology and more specific information on microbial dynamics that affect mineralization rates and soil organic matter turnover will become increasingly important agronomic inputs to synchronize plant N use.

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