

# Predicting Gross Nitrogen Mineralization and Potentially Mineralizable Nitrogen using Soil Organic Matter Properties

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Gross N mineralization is a fundamental soil process that plays an important role in determining the supply of soil inorganic N, highlighted by recent research demonstrating that plants can effectively compete with microbes for inorganic N. However, predictions of the supply of plant available N from soil have largely neglected gross N mineralization. As soil organic matter (SOM) is the substrate that microbes use in the process of N mineralization, characteristics of SOM fractions that are relatively easy to measure may hold value as predictors of gross N mineralization. To improve understanding of predictive relationships between SOM fraction properties and gross N mineralization, we assessed 32 measures of SOM quality and quantity, including physically, chemically, and biologically defined SOM fractions, for their ability to predict gross N mineralization across a wide range of soil types (Aridisols to Mollisols) and crop management systems (organic vs. inorganic based fertility) in Israel and the United States. We also assessed predictions of a commonly employed indicator of soil N availability, potentially mineralizable N (PMN, determined by 7-d anaerobic incubation). Organic fertility management systems consistently enhanced gross N mineralization and PMN compared with inorganic fertility management systems. While several SOM characteristics were significantly correlated with both gross N mineralization and PMN, other characteristics differed in their relationships with gross N mineralization and PMN, highlighting that these assays are controlled by different factors. Multiple linear regressions (MLR) were utilized to generate N mineralization predictions: five (gross N mineralization) or six (PMN) predictor models explained >80% of the variation in both gross N mineralization and PMN ( $R^2 > 0.8$ ). The MLR models successfully predicted gross N mineralization and PMN across diverse soil types and management systems, indicating that the relationships were valid across a wide range of diverse agroecosystems. The ability to develop predictive models that apply across diverse soil types can aid soil health assessment and management efforts.

Abbreviations: CW, cold water extractable; FI, Fluorescence indices; HIX, humification index;  $HIX_{ohno}$ , humification index from Ohno (2002); HW, hot water extractable; MLR, multiple linear regression; OM, organic matter; PMN, potentially mineralizable N; POM, particulate organic matter; PTFE, Polytetrafluoroethylene; SOM, soil organic matter; TW, total (cold + hot) water extractable; WHC, water-holding capacity.

## Core Ideas

- Gross N mineralization and PMN are related to different SOM properties.
- Multiple linear regressions generated predictions of N mineralization that were validated across diverse agroecosystems.
- Organic soil amendments consistently increased N mineralization.

Mineralization of organic N stored in SOM is a key agroecosystem process, often providing the majority of N taken up by crops (Stevens et al., 2005; Gardner and Drinkwater, 2009). Net N mineralization is assumed to represent the release of plant available N from organic N pools based on the premise that plants only access the supply of mineralized N that is produced in excess of microbial demand (e.g., Drinkwater and Snapp, 2007). Based on this assumption, much research has focused on the development of predictors of potential net N mineralization (e.g., Ros et al., 2011a, 2011b). Potentially mineralizable N, determined by a relatively short anaerobic incubation, is one potential indicator

This article has supplemental material.

Soil Sci. Soc. Am. J. 81:1115–1126

doi:10.2136/sssaj2017.02.0055

Received 16 Feb. 2017.

Accepted 8 June 2017.

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of net N mineralization and crop yield response to N fertilizer (Keeney and Bremner, 1966; Nyiraneza et al., 2012). However, there has only been limited success in applying PMN and other indicators of net N mineralization to the development of N fertilizer recommendations that optimize crop yields and minimize N losses (Sawyer et al., 2006).

Gross N mineralization is the absolute production of  $\text{NH}_4^+$  from SOM-N due to microbial activity, in contrast to net N mineralization which is the difference between gross N mineralization and inorganic N consumption processes (e.g., microbial N immobilization, denitrification) in the absence of plant uptake. As net N mineralization is determined by multiple N-producing and N-consuming processes, it does not represent the true N mineralization capacity (i.e., gross N mineralization) and is typically only weakly correlated with gross N mineralization (Wang et al., 2001; Schimel and Bennett, 2004; Booth et al., 2005). Gross N mineralization may hold promise for supplying novel information about the potential soil N supply in agricultural systems as there is evidence from both unmanaged and agricultural ecosystems that plants can successfully compete with microbial immobilization (Kaye and Hart, 1997; Inselsbacher et al., 2010). However, compared with net N mineralization, gross N mineralization remains difficult to measure and poorly understood in agricultural systems, and predictions of gross N mineralization are relatively uncommon.

Predictions of gross N mineralization are of interest in agroecosystems because this fundamental N cycling process underlies N losses and crop production. Plants growing in soils with relatively high rates of gross N mineralization but small inorganic N pools, such as many forest soils, must compete with microbes for inorganic N to meet their N needs (Kaye and Hart, 1997). Agroecosystems managed to approach such a condition could offer a means to greatly reduce environmental N losses that are associated with large inorganic N pools. Accurate and precise predictions of gross N mineralization rates could identify management practices that enhance gross N cycling rates, and thus contribute to the design of agricultural systems that are effective in conserving N for improved environmental performance (Robertson and Swinton, 2005; Davidson et al., 2012).

The quantity of operationally defined SOM fractions can provide information regarding the abundance of substrates for N mineralization, while the quality of SOM fractions may relate to the availability of specific pools for microbial decomposition, as well as to the stoichiometry of microbes and their N demand (Wander, 2004). Labile SOM fractions consist of heterogeneous materials, and can represent both the substrate and the by-products of SOM decomposition and N mineralization (Ros, 2012). While SOM fractions do not directly represent functional SOM

pools, they can still prove to be useful representations of the status of labile SOM (Wander, 2004; von Lützow et al., 2007). Correlations between the quantity and quality of SOM fractions, net N mineralization and PMN have been investigated in multiple attempts to develop predictors of net N mineralization (Sharifi et al., 2007; Schomberg et al., 2009; Ros et al., 2011b; Ros, 2012). While gross N mineralization has also been shown to correlate positively with overall SOM quantity in agroecosystems (Booth et al., 2005; Herrmann and Witter, 2008), assessments of the ability of multiple SOM fractions to generate predictions of gross N mineralization are relatively rare (see Wang et al., 2001). Additionally, in contrast with gross N mineralization, PMN integrates both gross  $\text{NH}_4^+$  production and  $\text{NH}_4^+$  consumption processes that may have distinct relationships with SOM characteristics. Thus, SOM characteristics may relate differently to gross N mineralization and PMN (Wang et al., 2001).

In addition to conventional quantitative measures of SOM fractions, fluorescence techniques have been developed to rapidly measure chemical qualities of SOM fractions, and may prove to be effective predictors of N mineralization. The fluorescence properties of cold- or hot-water-extractable SOM describe chemical qualities of certain fractions of labile SOM that may be related to the susceptibility of SOM to microbial degradation and N mineralization (Parlanti et al., 2000; Rinot, 2011). Multiple fluorescence indices (FIs) have been developed (Table 1) that may complement more common characterizations of C and N in SOM fractions. However, predictive relationships between FIs and gross N mineralization or PMN remain poorly characterized.

Identification of soil properties that can effectively predict gross N mineralization and PMN across diverse environments is important for increasing understanding of N mineralization. Edaphic conditions can influence the relationship between SOM properties and N mineralization potential (Nyiraneza et al., 2012), and fertility management strategies may similarly influence such relationships as they can cause changes in both SOM characteristics and N mineralization potential, particularly over long time scales. Organic N amendments, for example, can affect both the concentration and quality of labile organic matter (OM) fractions (Wander et al., 1994; Chantigny, 2003; Wander, 2004; Gregorich et al., 2003). A previous meta-analysis found a large degree of uncertainty in predictions of net N mineralization across multiple experiments (Ros, 2012). Thus, identification of predictors that are applicable across soil types and management strategies requires the assessment of predictive relationships from a variety of distinct soil types under diverse fertility management strategies.

**Table 1. Details of fluorescence spectroscopy indices used in this study.**

Fluorescence Index	Excitation wavelength	Formula	Reference
Humification Index	254	emission @ 435–480/emission @ 300–345	Zsolnay et al., 1999
Humification Index (Ohno)	254	emission @ 435–480/(emission @ 300–345 + emission at 435–480)	Ohno, 2002
Fluorescence Intensity	370	emission @ 470/emission @ 520	McKnight et al., 2001
Freshness Index	310	emission @ 380/maximum emission between 420 and 435	Parlanti et al., 2000

The development of easily measurable predictors of gross N mineralization and PMN holds promise for advancing understanding of N cycling in agroecosystems. In this study, we analyzed soil samples from six long-term agricultural experiments in Israel and the U.S. Midwest with widely varying soil, climatic, and management contexts to examine the robustness of predictive relationships between both potential gross N mineralization and PMN and a suite of biological and chemical properties of SOM quantity and quality. We tested three hypotheses: 1. The same SOM quantity and quality properties will predict both gross N mineralization and PMN; 2. Predictive relationships between SOM properties and gross N mineralization and PMN will not differ across widely varying agricultural soils from the US Midwest and Israel; and 3. Soil OM predictors of gross N mineralization and PMN will not differ across agricultural systems using both organic and inorganic fertility management strategies.

## MATERIALS AND METHODS

Soils from six long-term cropping systems experiments in Israel and the US Midwest were evaluated in this study. Treatments in each experiment consisted of cropping systems that differed in crop rotation and N fertility management (Table 2). Nitrogen fertility management was classified as either organic or inorganic. Organic management comprised any use of organic N fertility sources including animal manures, green manures, compost, and sludge. Inorganic management consisted of use of synthetic N fertilizers or treated wastewater with high concentrations of inorganic N. Soils were sampled (0- to 20-cm depth) in the fall of 2014. More than 20 soil cores (1.8-cm diam.) were taken from each experimental plot and composited to create a representative soil sample. A total of 59 plots from 21 cropping systems were sampled. Soil samples were air-dried at 20°C and sieved to 2 mm prior to further analysis.

## Nitrogen Mineralization

Potential rates of gross N mineralization were measured at soil moisture content and temperature levels favorable for microbial activity using the  $^{15}\text{N}$  pool dilution technique (Hart et al., 1994). Two duplicate 25-g soil samples, previously air-dried and sieved as described above, were moistened to 60% water holding capacity (WHC) and pre-incubated at 23°C for 24 h. Following pre-incubation, 1.0 mL of  $\text{NH}_4\text{Cl}$  solution (100 mg N  $\text{L}^{-1}$ , 7.5% atom percent excess)

**Table 2. Location, selected soil properties, and general management of the six experimental sites used in this study. All soils were sampled in the fall of 2014.†**

Experiment	Year established	Location	Soil texture class	CEC	Soil pH	SOC	Crop rotations	N fertility strategy & source
Marsden	2002	Iowa USA 42°01' N, 93°47' W	Loam	18.2	7.2	27.3	Corn-soybean	I: UAN O: UAN + composted manure
VICMS	1989	Minnesota, USA 44°15' N, 95°19' W	Clay loam	18.5	7.5	23.9	Corn-soybean-oat/alfalfa-alfalfa Corn-soybean Corn-soybean	I: Urea, MAP, DAP, UAN I: Urea, MAP, DAP, UAN I: Urea, MAP, DAP, UAN
WICST	1990	Wisconsin, USA 43°20' N, 89°21' W	Silt loam	16.3	6.5	24.6	Corn-soybean-oat/alfalfa-alfalfa Corn-soybean-oat/alfalfa-alfalfa Corn-soybean Corn-soybean-wheat/clover	O: Liquid swine manure, cattle manure I: UAN O: Pelleted poultry manure
Yakum	2000	Yakum, Israel 32°15' N 34°50' E	Sandy loam	12.4	7.0	12.2	Corn-oat/alfalfa-alfalfa Avocado Persimmon	O: Dairy manure O: Multiple organic fertility sources including treated waste water I: Treated waste water + inorganic N
Revadim	2011	Revadim, Israel 31°46' N 34°49' E	Clay	32.1	7.4	14.1	Uncultivated Corn-wheat Corn-wheat Corn-wheat Corn-wheat	NI: No fertilizer NI: Digested sludge + inorganic N NI: Composted sludge + inorganic N NI: N-Viro + inorganic N
Gilat	2009	Gilat, Israel, 31°19' N 34°38' E	Loam	16.8	7.6	17.7	Corn-wheat Clover-corn-wheat-potato-corn-wheat Clover-corn-wheat-potato-corn-wheat Clover-corn-wheat-potato-corn-wheat Clover-corn-wheat-potato-corn-wheat	NI: N-Viro NI: No fertilizer O: Compost O: Compost O: Compost I: Urea

† I, inorganic N management; O, organic fertility management; NI, not included in fertility management analysis; UAN, urea ammonium nitrate; MAP, monoammonium phosphate; DAP, diammonium phosphate; N-Viro, alkaline stabilized sludge (see Logan and Harrison, 1995). Further management information can be found for the experiments: Marsden, Liebman et al. (2008) and Davis et al. (2012), VICMS, Coulter et al. (2011), WICST, Posner et al. (2008) and Jokela et al. (2011).

was injected into each of the two soil samples using a side-port needle in 10 ~0.1-mL injections. After 10 min, one of the duplicate samples was extracted with 2 mol L<sup>-1</sup> KCl, and the second duplicate was extracted after incubation for 24 h at 23°C. Ammonium content of the extracts was determined by colorimetry (Hood-Nowotny et al., 2010), then the NH<sub>4</sub><sup>+</sup> in the extracts was diffused on polytetrafluoroethylene (PTFE) enveloped acidified filter paper discs by raising the pH above 10 using MgO (Stark and Hart, 1996). Isotope ratio mass spectrometry was used to determine <sup>15</sup>N enrichment of the acidified filter paper discs, and the equations of Kirkham and Bartholomew (1954) were used to calculate gross N mineralization rates.

A 7-d anaerobic incubation was used to determine PMN (Schomberg et al., 2009). Prior to incubation, the preexisting soil NH<sub>4</sub><sup>+</sup> concentration was determined by shaking 5.0 g of soil with 40 mL of 2 mol L<sup>-1</sup> KCl solution for 1 h, and filtration through Whatman No. 42 filter paper. A duplicate 5.0-g soil sample was submerged under 20 mL of deionized water and incubated at 40°C for 7 d, after which the soils were resuspended and extracted with 20 mL of 4 mol L<sup>-1</sup> KCl. Both pre- and post-incubation NH<sub>4</sub><sup>+</sup> concentrations were determined by colorimetry to determine the total change in NH<sub>4</sub><sup>+</sup> (Hood-Nowotny et al., 2010). Potentially mineralizable N was calculated as the average daily rate of change in NH<sub>4</sub><sup>+</sup>-N concentration over the course of the 7-d incubation.

### Soil Organic Matter Properties

A suite of 38 SOM properties (Table 3) was measured for soil samples collected from each experimental unit at each site. Total soil C and N were determined by dry combustion elemental analysis in a LECO Truspec CHN analyzer (LECO Corp. St. Joseph MI). Particulate organic matter (POM), commonly considered a labile fraction of SOM, was separated from non-particulate organic matter (Non-POM) by wet-sieving (Cambardella and Elliot, 1992; Wander, 2004). Briefly, POM between 2.0 and 0.53 mm in size was collected after a 2.0-g soil sample was dispersed and passed through a 0.53-mm sieve under a stream of water and the material retained on the sieve considered the POM fraction. Soil material passing through the filter was collected as the non-POM fraction. Particulate OM and non-POM samples were dried at 50°C, weighed, and C and N contents were determined by dry combustion as above.

Soil respiration on rewetting (CO<sub>2</sub> burst) has been proposed as an index of microbially available SOM, and in this study was determined over 3-d and 7-d periods (Franzluebbers et al.,

2000). An 8.0-g sample of air-dried and 2-mm sieved soil was weighed into a cup and placed in a 0.25-L Mason jar. The jar lid was equipped with a sampling septum. The soil sample was wetted to 60% WHC, and the jar was immediately capped and incubated at 23°C. Carbon dioxide concentrations in the headspace were determined by infrared gas analysis with a LICOR 7000 CO<sub>2</sub> analyzer (LI-COR Biosciences, Lincoln NE).

Organic matter extracted by cold and hot water has been utilized as a measure of highly labile SOM (Ghani et al., 2003). In this study, cold and hot water extractable SOM was extracted sequentially (Rinot, 2011). First, cold water extractable (CW) OM was removed by shaking 5.0 g of soil with 35 mL of 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> solution for 1 h, centrifuging at 1053 × *g* for 20 min, and then vacuum filtering the supernatant through 0.45-μm PTFE filters. The residual soil sample was then extracted with 35 mL of 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> at 80°C for 4 h under gentle agitation, and centrifuged and filtered as described for the CW extract to extract the hot water (HW) extractable OM. Water extracts were acidified to pH ~3 to remove inorganic C and frozen until analysis. Inorganic N concentrations in the water extracts were determined by colorimetry (Hood-Nowotny et al., 2010), and dissolved organic C and N were determined with a Shimadzu TOC-L analyzer equipped with a TN unit (Shimadzu, Kyoto Japan). Two replicates of each soil were analyzed for CW and HW C and N, as well as fluorescence measurements. The resulting replicate values were averaged and the soil means were used for all statistical analyses. Cold water extractable and HW total N was calculated as the sum of organic N and inorganic N in the CW and HW extracts.

Fluorescence of the CW and HW extracts was determined using an Aqualog fluorometer (HORIBA Scientific, Kyoto Japan) and 1-cm path-length quartz cuvettes. Excitation-emission matrices (wavelengths 240–600 nm) were generated at 3.28-nm excitation intervals with a 1-s integration time. Samples were diluted with ultrapure water to ensure sample absorbance values at 254 nm did not exceed 0.1 cm<sup>-1</sup> to avoid inner filter effects (Borisover et al., 2012). Raman unit normalization of the spectra was performed by dividing each spectrum by the Raman peak integration area that was recorded for each analysis session. The fluorescence signal of a blank standard of ultra-pure water was then subtracted from the sample signal. The humification index, Ohno's modified humification index, fluorescence intensity, and freshness index were calculated from sample excitation–emission matrices according to the formulas in Table 1.

**Table 3. Measured soil organic matter (SOM) quantity and quality parameters.**

SOM fraction	Measured properties	Derived properties
Total soil	Total C, Total N	Total C/N
Particulate organic matter (POM)	POM-C, POM-N	POM C/N, POM-C/Total C
Non-particulate organic matter	non POM-C, non-POM-N	non POM C/N
Cold water dissolved organic matter	CW C, CW ON, CW NO <sub>3</sub> <sup>-</sup> , CW NH <sub>4</sub> <sup>+</sup> , Fluorescence	CW total N, CW C:ON, Fluorescence indices
Hot water dissolved organic matter	HW C, HW ON, HW NO <sub>3</sub> <sup>-</sup> , HW NH <sub>4</sub> <sup>+</sup> , Fluorescence	HW total N, HW C:ON, Fluorescence indices
Total water dissolved organic matter	TW OC, TW ON	
Readily respired OC: 3-d and 7-d	0 to 3 d CO <sub>2</sub> , 3 to 7day CO <sub>2</sub>	7 d CO <sub>2</sub> :Total C



## Statistical Analyses

Gross N mineralization and PMN data were visually checked for approximate normality and transformations were not considered required. Site and N management effects on gross N mineralization and PMN were analyzed by ANOVA. Site (i.e., location of the six experiments) and N management (inorganic vs. organic) were considered as fixed effects. Treatments were classified as inorganic if they received exclusively inorganic N fertilizers, and were considered organic if they received organic N amendments such as animal manure, green manure, compost, or sludge. For this analysis, the Revadim site (Table 2) was not included because a treatment receiving only inorganic N fertilizer was not available. Simple linear correlations between N mineralization and SOM quantity and quality measurements across all sites were investigated by pairwise correlations using Pearson's  $r$  statistic. Because of the large number of comparisons ( $n = 38$ ) in this analysis we employed a Bonferroni adjustment to  $\alpha$  ( $\alpha = 0.05/38 = 0.0013$ ) to control the type I error rate, in which correlations with  $p < 0.0013$  were considered statistically significant.

Stepwise MLR were performed using proc GLMSELECT in SAS software (SAS Institute, Inc., Cary, NC), with k-fold cross validation utilizing predicted residual sum of squares (PRESS) as the model selection criterion. This procedure identifies the MLR model composed of the combination of predictor variables with the minimum PRESS score. The performance of the selected MLR models was examined by comparing observed values of gross N mineralization or PMN to predicted values using regression analysis. Predictions were calculated using model equations in the form of  $y = \beta_0 + \beta_1 X_1 + \dots + \beta_n X_n$  where  $y$  was gross N mineralization or PMN,  $\beta_0$  was the intercept and  $\beta_1 \dots \beta_n$  were parameter estimates, and  $X_1 \dots X_n$  were the measured SOM variables.

Potential differences between MLR predictions for sites and N management regimes were investigated by analysis of covariance is SAS proc MIXED, which tests differences in intercepts and slopes of the regression lines for each category. The robustness of the MLR model across the experimental sites and management categories was further evaluated by systematically excluding a single category from the dataset, and then parameterizing the MLR model with the reduced dataset. The resulting parameterized model was then used to predict gross N mineralization or PMN in the excluded site, and the predictions were compared with the observed data using regression analysis in SAS proc GLM to validate the model for the "unknown" site or management category.

The MLR techniques can be susceptible to issues with multicollinearity, wherein the variance explained by individual predictors overlaps to a large degree, thus disrupting the estimation

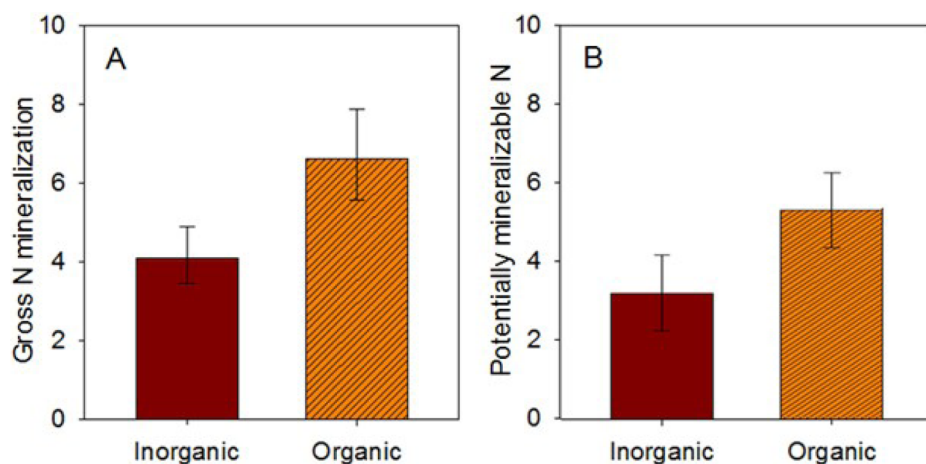
and interpretation of regression coefficients (Graham, 2003). However, multicollinearity does not preclude examination of the model's utility for making predictions (Graham, 2003). Multicollinearity among the selected parameters in the MLR models was analyzed by considering variance inflation factors (VIF), where  $VIF = 1/(1-R^2)$  and  $R^2$  is the coefficient of determination when the parameter in question is regressed against the other parameters in the model. A  $VIF > 10$  was considered an indicator of significant multicollinearity (Izenman, 2008). All statistical analyses were performed in SAS 9.3 (SAS Institute Inc., Cary NC).

## RESULTS

The diverse soils employed in this study generated a wide range of potential gross N mineralization and PMN rates: gross N mineralization rates ranged from 2.08 to 12.6 mg N kg soil<sup>-1</sup> d<sup>-1</sup>, while PMN ranged from 0.20 to 14.5 mg N kg soil<sup>-1</sup> d<sup>-1</sup>. The organic fertility management systems had greater rates of gross N mineralization ( $p = 0.002$ ) and potential N mineralization ( $p = 0.004$ ) than the inorganic fertility management systems (Fig. 1). Additionally, gross N mineralization differed significantly among the six sites ( $p = 0.002$ ), while PMN did not differ among sites ( $p = 0.23$ ). Site by management system interactions were not significant for gross N mineralization ( $p = 0.65$ ) or for PMN ( $p = 0.52$ ). Gross N mineralization and PMN were positively but weakly correlated ( $r = 0.38$ ,  $p = 0.003$ , Fig. 2).

A large number of significant predictors of gross N mineralization and PMN were different: although 9 of the 38 predictors demonstrated significant correlations ( $p < 0.05$  after Bonferroni correction) with both gross N mineralization and PMN, 16 predictors were significantly correlated with either gross N mineralization or PMN, and 13 predictors were uncorrelated with both N mineralization assays (Table 4). In general, standardization of C and N fractions by the total soil C and N (e.g., g fraction C g total soil C<sup>-1</sup>) did not improve correlations.

Several measures of the C and N content of labile fractions of SOM were positively correlated with both gross N mineraliza-



**Fig. 1. Nitrogen mineralization in treatments with organic fertility management compared with inorganic fertility management for (A) gross N mineralization and (B) potentially mineralizable N. Bars represent the average of five sites. Units are mg N kg<sup>-1</sup> soil d<sup>-1</sup>, error bars are 95% CI.**

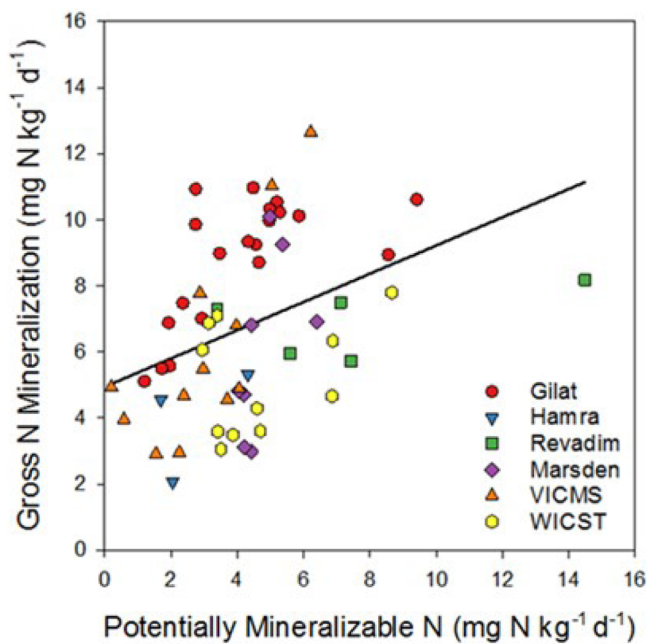


Fig. 2. Linear correlation between potentially mineralizable N and gross N mineralization across six sites. Pearson's  $r = 0.38$ .

tion and PMN (Table 4). For example, POM C, HW C, and HW N correlated positively with both gross N mineralization and PMN. Total water OC (CW + HW OC) was positively correlated with gross N mineralization and PMN, but correlations were not as strong as those for HW C alone. Nitrate concentrations in the HW and CW extracts were also positively correlated with both gross N mineralization and PMN.

Other predictors were related to either gross N mineralization or PMN, but not both (Table 4). The  $\text{CO}_2$  burst measurements were positively correlated with PMN but not gross N mineralization, while both non-POM C and non-POM C:N were positively correlated with gross N mineralization but not PMN. Among the FIs, the humification indices (HIX and  $\text{HIX}_{\text{Ohno}}$ ) of the CW and HW extracts were significantly positively correlated with gross N mineralization. No FIs were significantly correlated with PMN.

Multiple linear regression models accounted for >80% of the variation in observed both gross N mineralization and PMN (Table 5, Table S1). The MLR model selected for gross N mineralization included five predictors: non-POM C, CW organic N, HW  $\text{NO}_3^-$ , TW extractable organic C, and CW HIX (Fig. 3, Table 5), with a resulting  $R^2$  of 0.82 and RMSE of  $1.16 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ . The PMN MLR model included six different predictor variables: POM-N normalized to total N, non-POM N, CW total N, HW C, HW total N, and HW  $\text{NH}_4^+$ , with the resulting model achieving an  $R^2$  of 0.80 and RMSE of  $1.11 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$  (Fig. 3, Table 5). The VIFs of model parameters suggested slight multicollinearity in our PMN model between the predictors HW organic C and HW total N (Table 5, Table S1).

The MLR prediction equations developed here were valid across site and management factors. Site did not influence the relationship between predictions and observations for either

Table 4. Pearson correlation coefficients between gross N mineralization and potential N mineralization and soil organic matter properties tested as potential predictors.

Predictor†	Gross N mineralization $r$	Potential N mineralization $r$
Total C	-0.16	0.16
Total N	-0.14	0.16
Total C/N	-0.25	-0.02
POM C	0.66*	0.43*
POM N	0.43*	0.36
POM C/N	-0.01	-0.09
POM C/Total C	0.53*	0.05
POM N/Total N	0.42*	0.09
Non-POM C	0.58*	0.23
Non-POM N	-0.12	0.13
Non-POM C/N	0.41*	-0.05
$\text{CO}_2$ burst 0–3 d	-0.02	0.55*
$\text{CO}_2$ burst 4–7 d	0.23	0.66*
$\text{CO}_2$ burst 0–7 d	0.08	0.62*
$\text{CO}_2$ burst 0–7 d/Total C	0.34	0.10
CW OC	0.49*	0.39
CW $\text{NO}_3^-$	0.58*	0.54*
CW $\text{NH}_4^+$	0.06	0.21
CW ON	0.22	0.49*
CW total N	0.56*	0.61*
CW OC:ON	-0.09	-0.20
HW OC:ON	-0.60*	-0.38
HW OC	0.45*	0.64*
HW $\text{NO}_3^-$	0.59*	0.59*
HW $\text{NH}_4^+$	-0.12	0.20
HW ON	0.57*	0.69*
HW total N	0.56*	0.75*
TW OC	0.52*	0.66*
TW ON	0.56*	0.72*
TW OC:ON	-0.47*	-0.40
CW HIX	0.66*	0.02
CW $\text{HIX}_{\text{Ohno}}$	0.64*	0.04
CW FI	0.34	0.25
CW freshness index	0.14	0.32
HW HIX	0.53*	0.17
HW $\text{HIX}_{\text{Ohno}}$	0.52*	0.09
HW FI	0.09	0.24
HW freshness index	-0.24	0.13

\* significant at a level of 0.05 after Bonferroni correction.

† CW, cold water; FI, fluorescence indices; HIX, humification indices;  $\text{HIX}_{\text{Ohno}}$ , humification indices from Ohno (2002); HW, hot water; OC, organic C; ON, organic N; TW, total water (CW + HW).

gross N mineralization (slopes different  $p = 0.13$ , intercepts different  $p = 0.17$ , Fig. S1) or PMN (slopes different  $p = 0.68$ , intercepts different  $p = 0.73$ , Fig. S1). Cross validation of the gross N mineralization predictions for each site, in which the MLR model was parameterized with a single site excluded from the dataset and then used to predict gross N mineralization in that site, revealed agreement between predictions and observations where predicted gross N mineralization values were within 20% of the measured value in 69% of samples, and predictions were within 35% of observed values for 88% of samples. The gross

**Table 5. Multiple linear regression models for prediction of gross N mineralization and potential N mineralization. N mineralization predictions were calculated by multiplying parameter estimates and measured values for SOM variables.**

Dependent	Variable	Parameter estimate	Standard error	Pr >  t	VIF†	Semi-partial $R^2$	$R^2$ Model
Gross N mineralization	Intercept	-4.53	0.845	<0.0001			0.815
	Non-POM C	0.179	0.030	<0.0001	1.2	0.33	
	CW ON	-0.171	0.069	0.017	1.4	0.03	
	HW NO <sub>3</sub> <sup>-</sup>	0.716	0.313	0.026	2.1	0.14	
	TW (cold + hot) OC	0.012	0.003	<0.0001	1.7	0.07	
	CW HIX	0.255	0.030	<0.0001	1.1	0.24	
Potential N mineralization	Intercept	3.93	6.19	0.53			0.804
	POM N/Total N	-293	83.5	0.001	3.3	0.01	
	Non-POM N	9.57	3.19	0.0042	4.3	0.09	
	CW total N	0.288	0.155	0.069	5.6	0.52	
	HW OC	-0.366	0.0657	<0.0001	19.1	0.01	
	HW total N	2.56	0.389	<0.0001	26.3	0.17	
	HW NH <sub>4</sub> <sup>+</sup>	0.568	0.392	0.15	1.1	0.01	

† CW, cold water; HIX, humification indices; HW, hot water; OC, organic C; ON, organic N; TW, total (cold + hot) water; VIF, variance inflation factor.

N mineralization model performed well for four of the six sites where RMSE ranged from 0.35 to 0.90 mg N kg<sup>-1</sup> soil d<sup>-1</sup>, but at the Marsden site RMSE = 1.21 mg N kg<sup>-1</sup> soil d<sup>-1</sup> and at the WICST site there was worse agreement between observations and predictions with RMSE = 1.64 mg N kg<sup>-1</sup> soil d<sup>-1</sup> (Fig. 4). The estimates for slopes and intercepts of regressions of observed and predicted gross N mineralization were not significantly different ( $p > 0.05$ ) from 0 and 1, respectively, at four of the six sites, but at WICST and Hamra the slope estimate was not significant ( $p > 0.05$ ) so it could not be tested for its difference from 1. The Hamra site was limited by a small sample size (three observations) and was difficult to assess.

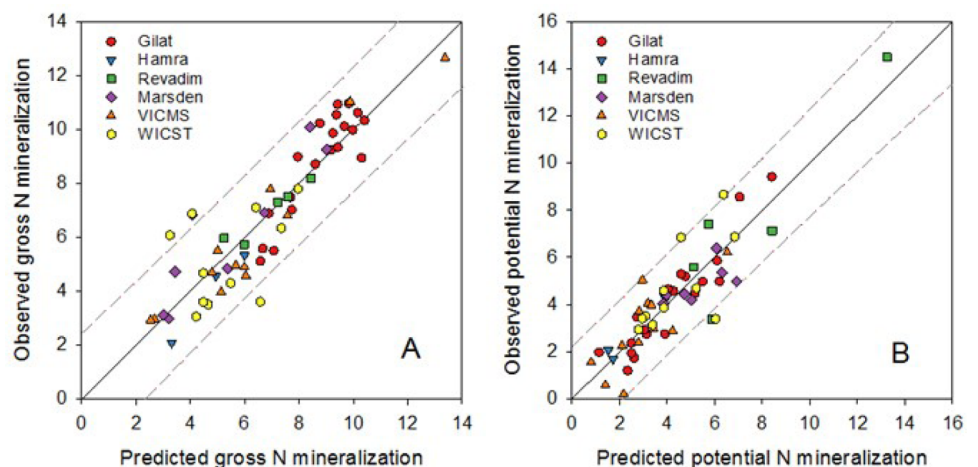
The relationships between the observed N mineralization potentials and those predicted by MLR were not different in the two N fertility management categories (organic v. inorganic N) for gross N mineralization (slopes different  $p = 0.88$ , intercepts different  $p = 0.39$ , Fig. S2) or PMN (slopes different  $p = 0.63$ , intercepts different  $p = 0.93$ , Fig. S2). Thus, N mineralization was not systematically over or under predicted in either management strategy, and fertilizer management did not alter the relationships between SOM predictors and gross N mineralization or PMN. Cross validation over the management categories revealed that the predictions matched observations with moderate accuracy, with RMSE = 1.22 mg N kg<sup>-1</sup> soil d<sup>-1</sup> for both inorganic and organic management classes. Regression slopes for predicted vs. observed gross N mineralization were not significantly different from 1 ( $p > 0.05$ ) and the

intercepts were not significantly different from 0 ( $p > 0.05$ ) for either management category (Fig. 5).

## DISCUSSION

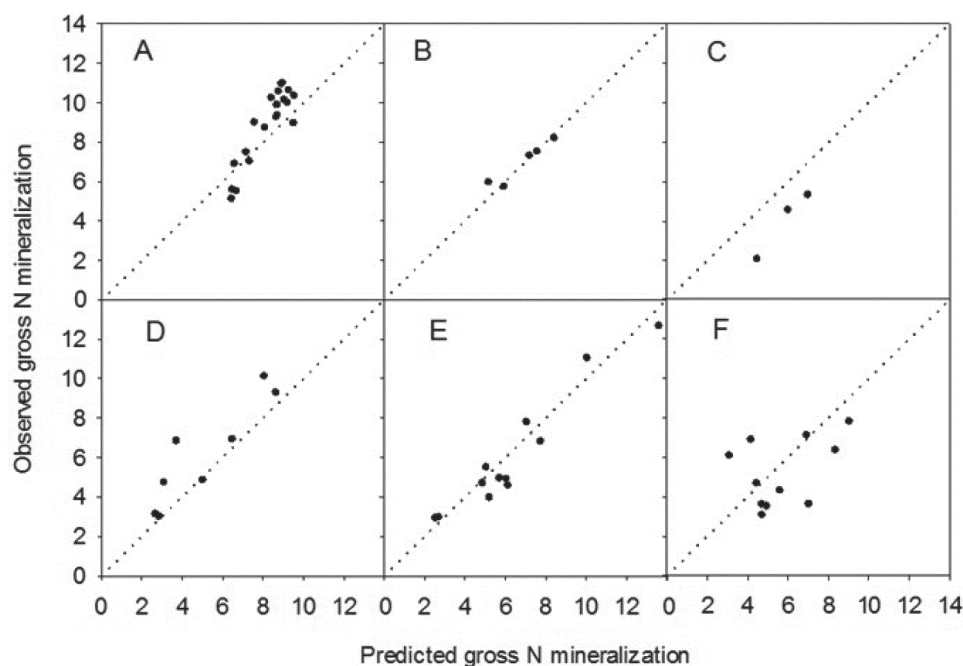
### Simple Correlations

The majority of SOM properties with a significant linear relationship with gross N mineralization differed from those with a significant relationship with PMN, leading us to reject Hypothesis 1. This result suggests that the processes measured by these assays are controlled by distinct factors. Aerobic net N mineralization and PMN have been shown to correlate closely (Gale and Gilmour, 1988), so the weak relationship between gross N mineralization and PMN rates in this study supports the distinct nature of gross and net N mineralization rates previously illustrated by a meta-analysis of gross and net N mineralization from both unmanaged and managed ecosystems (Booth et al.,



**Fig. 3. Multiple linear regression predictions vs. observations for (A) gross N mineralization and (B) potentially mineralizable N across six sites.  $R^2$  was 0.82 and 0.80 for gross and net mineralization models, respectively. The predictors selected for the gross N mineralization model were: Non-POM C, CW ON, HW NO<sub>3</sub><sup>-</sup>, TW OC, and CW HIX. Predictors selected for the potential N mineralization model were: POM-N/Total N, Non-POM N, CW total N, HW OC, HW total N, and HW NH<sub>4</sub><sup>+</sup>. Non-POM, non-particulate organic matter; CW, cold water; ON, organic N; HW, hot water; TW, total water (CW + HW); OC, organic C; HIX, humification index; POM, particulate organic matter. Solid lines are the 1:1 regression line, and dashed lines are 95% prediction intervals. Units are mg N kg<sup>-1</sup> soil d<sup>-1</sup>.**





**Fig. 4.** Correlations between the predicted and observed gross N mineralization from cross validation of the MLR model using sites as validation datasets. Predicted sites are (A) Gilat, (B) Revadim, (C) Hamra, (D) Marsden, (E) VICMS, and (F) WICST. Dotted lines show a 1:1 relationship.

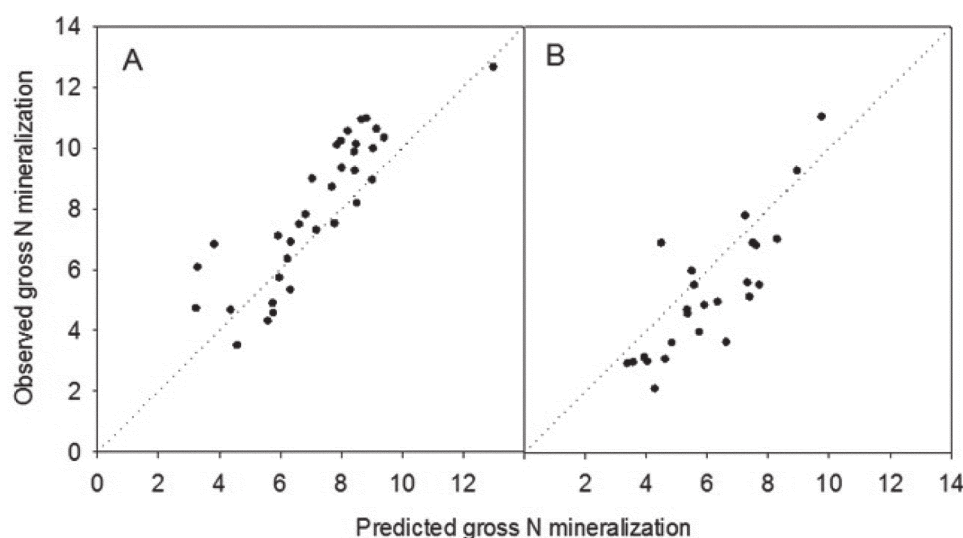
2005). Our results imply that gross N mineralization cannot be readily inferred from PMN and thus it will be necessary to develop specific prediction models for gross N mineralization, should research continue to emphasize the importance of gross N mineralization for plant N uptake (e.g., Kaye and Hart, 1997; Inselsbacher et al., 2010).

The positive correlations between labile SOM fractions and assays of N mineralization potential in this study support the previously reported relationships between labile SOM fractions and N mineralization potential in agricultural soils (Sharifi et al., 2007; Schomberg et al., 2009; McDonald et al., 2014). Labile SOM fractions are composed of heterogeneous mix-

tures of compounds with varying residence times, and components of these fractions serve as substrates for microbial activity or indicate the abundance of microbial metabolites (Wander, 2004; von Lützow et al., 2007). Thus, while correlations between properties of SOM fractions and N mineralization do not necessarily indicate causation they can still be useful indicators of the actual relationships between SOM properties and N mineralization (Ros, 2012).

Recent research focused on N mineralization suggests that gross N mineralization will be correlated with the size of labile SOM fractions closely related to microbial activity, while net N mineralization may correlate more closely with characteristics related to the C/N stoichiometry of SOM relative to microbial requirements for growth and respiration (Franzluebbers et al., 2000; Robertson and Groffman, 2007; Manzoni et al., 2012). The lack of correlation between gross N mineralization and CO<sub>2</sub> respiration across the agroecosystems examined here was surprising, as such a correlation was previously observed in forest ecosystems (Bengtsson et al., 2003), and suggests that carbon-use efficiency (CUE) of microbes differed across the experimental sites. Site-specific SOM characteristics could account for the lack of a consistent relationship between CO<sub>2</sub> respiration and gross N mineralization, as specific characteristics of microbially utilized SOM, such as C/N and chemical recalcitrance, can impact microbial CUE (Manzoni et al., 2012), weakening the relationship between CO<sub>2</sub> respiration and N mineralization.

Fluorescence characterization, as a measure of the quality of water extractable SOM, provided useful information for predicting gross N mineralization but not PMN. The positive correlations between the CW and HW HIX and gross N mineralization were unexpected as a previous study demonstrated that the biodegradability of dissolved OM extracted from forest and agricultural soils decreased with greater HIX, presumably indicating that organic compounds with a high HIX were highly processed or chemically complex and thus less labile (Kalbitz et al., 2003). However, dissolved



**Fig. 5.** Correlations between the observed gross N mineralization and the predicted gross N mineralization from cross validation using management classes for (A) organic management and (B) inorganic management. Dotted lines show a 1:1 relationship.



OM from cropland soils is typically already highly processed because of rapid OM decomposition (Toosi et al., 2012), so that chemical differences observed by HIX may no longer indicate relative biodegradability. As SOM progresses toward highly processed compounds it becomes relatively enriched in N (Sollins et al., 2009). Indeed, in this study HIX was negatively correlated with the C/N ratios of the extracted OM (Table S1). This N-rich nature of water extracted OM indicated by a greater HIX could thus stimulate gross N mineralization rates. Additionally, we observed that HIX was positively correlated with the size of labile fractions of OM (POM and water extractable OM, Table S1), demonstrating that agricultural soils with large amounts of highly processed OM compounds can also contain large labile pools of OM. Fluorescence methods offer a relatively quick, inexpensive, and data rich method for characterization of dissolved OM and may be well suited to advanced data mining or machine learning efforts aimed at characterizing dynamic biological processes. Spectroscopic methods that directly assess whole soils may also prove useful for efficient prediction of N mineralization potential (Fystro, 2002; Kira et al., 2014). Additional research effort should be focused on understanding how this data can be used to improve our understanding of soil N and C cycling dynamics.

In contrast to gross N mineralization, the positive correlation between  $\text{CO}_2$  respiration and PMN suggests microbial activity can provide an index to net N mineralization in these agricultural systems (Franzluebbers, 2016). The lack of correlation between PMN and measures of SOM quality including the C/N ratio of SOM fractions and FIs of water extractable OM further suggests that PMN did not depend on SOM quality, but rather SOM quantity and associated microbial activity (Table S2, Ros, 2012). In the agricultural soils studied here, the best predictors of net N mineralization were thus more related to overall microbial activity rather than SOM quality.

Interestingly, non-POM C (i.e., mineral-associated C), which has been considered a relatively stable fraction of OM (Hassink, 1997; Kögel-Knabner et al., 2008), was strongly correlated with gross N mineralization. This positive correlation suggests that non-POM C is not entirely composed of inaccessible organic compounds, but instead represents a significant source microbially available OM (Hall et al., 2015). Alternatively, this result may indicate an indirect effect of clay content on gross N mineralization, as non-POM C is expected to be much greater in fine-textured soils (Hassink, 1997). Indeed, in this study non-POM C concentration ( $\text{g non-POM C g}^{-1}$  soil) was highest at the experimental sites with high average soil clay content (data not shown).

Cold water  $\text{NO}_3^-$  and HW  $\text{NO}_3^-$  were highly correlated with both gross N mineralization and PMN. Few studies have specifically focused on water extractable  $\text{NO}_3^-$  as a direct indicator N mineralization, although  $\text{NO}_3^-$  extracted by salt solution has been extensively studied as an indicator of crop available N (Magdoff, 1991; Schröder et al., 2000). Cold water  $\text{NO}_3^-$  is likely highly variable over time and may be an indicator of the

abundance or quality of highly labile organic N, although it also may be related to the recent management legacy of the cropping systems or an indicator of the N saturation status of an agroecosystem. Hot water  $\text{NO}_3^-$  may result from the breakdown of labile organic N sources during the hot water extraction procedure and thus indicate the quantity of a labile organic N fraction, or alternatively may represent residual  $\text{NO}_3^-$  not extracted during the CW extraction.

## Fertility Management

Organic fertility management consistently increased both gross N mineralization and PMN across highly diverse soils and environments. Use of organic N sources in agricultural systems has been shown to enhance N cycling as well as N-use efficiency through the coupling of C and N cycles (Drinkwater and Snapp, 2007; Soon et al., 2007; Gardner and Drinkwater, 2009), and our results provide further evidence that additions of organic N fertilizers could be a universally effective approach for enhancing the supply of plant available inorganic N. As N mineralization activity is considered an important indicator of agricultural soil health (Haney et al., 2004; Idowu et al., 2009), use of organic N sources can be expected to enhance soil health in diverse agroecosystems. However, the risk of N loss to the environment could also be increased by greater N mineralization activity if it is not coupled with N immobilization or plant uptake, so practices aiming to increase N mineralization should also address the risk of N loss.

## Multiple Linear Regressions

The suite of selected measurements of SOM quality and quantity characteristics enabled the MLR models to consistently explain a large proportion of variation in N mineralization across diverse environments, which has been noted as a challenge in previous work (Wang et al., 2001; Ros, 2012). Multiple linear regression models were found to generate consistent predictions of gross N mineralization and PMN across widely varying agricultural soils from the US Midwest and Israel, supporting our second hypothesis. Similarly, we found support for our third hypothesis as the MLR predictions were shown to effectively capture the effects of diverse N management regimes on gross N mineralization and PMN, as correlations between observed and predicted values were similar across N management regimes. The cross validations presented here suggest that the MLR model could potentially perform well in predicting gross N mineralization for unknown sites, but a more robust evaluation of the model with independent datasets is needed to fully establish and refine this use.

The MLR model of gross N mineralization selected three predictors that were related to the size of various labile OM fractions: CW organic N, HW  $\text{NO}_3^-$ , and TW organic C. Inclusion of non-POM C as a predictor indicates that either the size of this OM fraction either directly explained variation in gross N mineralization, or that soil clay content indirectly explained variation. Finally, inclusion of CW HIX in the MLR model indicates

that the quality of CW SOM explained additional variation in gross N mineralization.

The MLR model for predicting PMN was mostly composed of the sizes of various labile OM fractions, including POM N normalized for total N, CW total N, HW  $\text{NH}_4^+$ , HW organic C, and HW total N. Similar to the gross N mineralization model the size of a relatively stable OM fraction (non-POM N) was included as a predictor in the model, indicating that either this OM fraction or the soil clay content was an important factor explaining variation in PMN.

Considered together, the simple correlations and MLR models suggest that gross N mineralization was closely related to several measures of SOM fraction size, thus reinforcing the importance of SOM quantity for gross N mineralization (Booth et al., 2005; Herrmann and Witter, 2008), but it was also closely related to several indicators of SOM quality. The size of SOM fractions were important predictors of PMN while SOM quality was relatively unimportant, in contrast to gross N mineralization providing further support for previous work suggesting SOM quality is of secondary importance to SOM quantity for predicting net N mineralization (Schomberg et al., 2009; Ros, 2012).

Ultimately SOM characteristics might be used to predict both gross N mineralization and PMN across diverse agroecosystems, thus informing management decisions such as N fertilizer use and cropping patterns. This study identified a set of relatively easy-to-measure SOM characteristics that generated predictions of gross N mineralization over a range of 2 to 12  $\text{mg N kg}^{-1} \text{ d}^{-1}$  with 95% confidence of  $\pm 2 \text{ mg N kg}^{-1} \text{ d}^{-1}$ . The anticipated use of N mineralization predictions will define the acceptable levels of uncertainty of such predictions. We concur with Ros (2012) that more mechanistic understanding of the relationship between SOM fractions and N mineralization will be important for continued development of useful predictors of N mineralization potential. Research efforts to develop applications of N mineralization predictions should also seek to better understand how laboratory-based predictions of potential rates of gross N mineralization and PMN relate to N dynamics in the field, including crop N uptake and yield response to N fertilizer.

## CONCLUSIONS

Soil fertility management strategy affected PMN and gross N mineralization across widely varying agricultural sites from the US Midwest and Israel, as treatments utilizing organic amendments demonstrated consistently greater rates of N cycling than inorganic treatments.

Multiple SOM characteristics were linked with either gross N mineralization or PMN, but only a small subset was related to both. Several predictors assessing the quantity of SOM fractions were related to both gross N mineralization and PMN, while the quality of SOM fractions was mainly related to gross N mineralization but not PMN. Among the FIs, the HIX was highly correlated to gross N mineralization.

Multiple linear regression equations used properties of diverse SOM fractions to predict gross N mineralization (se-

lected factors: non-POM C, CW organic N, HW  $\text{NO}_3^-$ , TW extractable organic C, and CW HIX) and PMN (selected factors: POM-N normalized to total N, non-POM N, CW total N, HW C, HW total N, and HW  $\text{NH}_4^+$ ), and were validated across widely varying agricultural soils from the US Midwest and Israel as well as across fertility management strategies. As the predictions of N mineralization were appropriate across a broad range of sites and fertility strategies, we believe that similar analysis techniques might be employed in diverse agroecosystems to improve estimates of soil N cycling rates and to assess soil quality and soil health. Predictions of gross N mineralization may lead to greater insights into N cycling activity, and could prove particularly relevant when combined with predictions of potential net N mineralization.

## SUPPLEMENTAL MATERIAL

Table S1 presents statistical characteristics of measured quantities; Table S2 presents correlations between predictor variables; Fig. S1 illustrates regression of MLR model predicted vs. observed N mineralization in the six experiments; and Fig. S2 illustrates regression of MLR model predicted vs. observed N mineralization in inorganic and organic N management systems.

## ACKNOWLEDGMENTS

Funding for this research was provided by the Iowa State University Department of Agronomy, the Frankenberger Professorship of Soil Science at Iowa State University, and the Bi-national Agriculture Research and Development Fund (US-4550-12).

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