Short- and Long-Term Labile Soil Carbon and Nitrogen Dynamics Reflect Management and Predict Corn Agronomic Performance

Steve W. Culman,* Sieglinde S. Snapp, John M. Green, and Lowell E. Gentry

ABSTRACT

Labile soil organic matter plays an extremely important role in crop nutrient acquisition, but quantifying this pool can be prohibitively expensive to farmers. A better understanding of rapid and inexpensive measures of labile organic matter could lead to new tools for predicting soil N supply and crop performance. Toward this end, we quantified several simple measures of labile C and N over the course of the corn ($Zea\ mays\ L$.) growing season in a long-term systems trial to determine:(i) the temporal dynamics of these measures, (ii) the long-term response of these measures to management, and (iii) the capacity of these measures to predict corn agronomic performance. We found that all labile soil measures (permanganate oxidizable carbon [POXC], C mineralization, N mineralization, and soil inorganic N) varied temporally and responded to long-term differences in management. Corn grain and vegetative biomass also responded to long-term treatment differences and these differences were strongly related to the measured labile soil C and N fractions. The history of crop rotation had a greater influence than management regime on all soil measures, with the exception of POXC. Of all the measures, C mineralization was the best predictor of agronomic performance both individually (r = 0.61-0.78, depending on corn stage), and when modeled with multiple indicators (six out of nine models). The results presented here demonstrate the strong relationship between crop growth and labile organic matter dynamics, and provide further evidence that C mineralization is an inexpensive, but sensitive predictor of corn agronomic performance.

Chighly reliant on soil organic matter dynamics, including the turnover of labile C and N, and the renewal of stabilized pools (Wander, 2004; Weil and Magdoff, 2004). These dynamics operate on short (seasonal) and long (years to decades) time scales, and understanding these dynamics is essential in moving toward more biologically-based cropping systems. Although soil organic matter is an extremely important indicator of overall soil quality, it can be insensitive to new management practices, as changes in total organic matter can take years to detect (Wander and Drinkwater, 2000; Wander, 2004).

The limitations of total organic matter as an indicator have led many researchers to focus on the labile pool of organic matter. This pool is small (typically <20% of the total), but pivotal to the rapid cycling of nutrients, soil aggregation, and C sequestration (Wander, 2004; Weil and Magdoff, 2004; Schmidt et al., 2011). Many measures of labile organic matter are sensitive and robust indicators of soil ecosystem change, but most are expensive to measure, and accordingly, are not offered

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by standard commercial soil testing laboratories (Phillips, 2010). There is a clear need for more inexpensive alternative measures of labile organic matter that enable farmers, extension educators, agronomists, and soil scientists to track and predict soil C and N dynamics in their fields.

Predicting soil N availability in cropping systems has been an ongoing challenge due to the complexities and interacting forces of weather, soil biology and physical properties, residue quality, and management practices (Cabrera et al., 2005; Schomberg et al., 2009). In a research setting, soil N availability is often predicted with laboratory incubations of soil, that is, N mineralization potential (Stanford and Smith 1972). The time and costs associated with this analysis has limited the adoption into most standard commercial soil testing laboratories, although some specialized labs do offer N mineralization tests (e.g., Idowu et al., 2008).

Instead of incubations, growers currently use two primary tools to predict soil N availability: the pre-sidress nitrate test (PSNT; Magdoff et al., 1984) and leaf chlorophyll content (Piekkielek and Fox, 1992; Scharf et al., 2006). The PSNT measures soil nitrate in corn at V4–V6 (Fox et al., 1989), while leaf chlorophyll content measures the greenness of early stage crop leaves relative to a highly-fertilized reference strip. Although both of these tests provide a prescriptive fertilizer recommendation, many growers do not use them for a variety of reasons (Markwell et al., 1995; Andraski and Bundy, 2002; Schmidt et al., 2009).

Another approach has been to approximate N mineralization potential by measuring short-term C mineralization (Franzluebbers et al., 2000). Carbon mineralization is a less expensive measurement than N mineralization potential,

Abbreviations: C-min, carbon mineralization; N-min, nitrogen mineralization potential; POXC, permanganate oxidizable carbon; PSNT, pre-sidedress nitrate test.

Table I. Corn N fertilizer application rates in 2011 and soil organic carbon (SOC) and total soil nitrogen (TSN) from 2008 in the long-term living field laboratory trial at the Kellogg Biological Station (n = 24).

| Management | Rotation† | Inorganic N fertilizer applied | N credit from organic sources | Total estimated N applied‡ | soc | TSN |
|--------------|-----------|--------------------------------|-------------------------------|----------------------------|------|------------------------|
| | | | kg N ha ^{-l} | | g kg | ^{-l} soil ——— |
| Conventional | CC | 151 | 0 | 151 | 0.68 | 0.075 |
| | CSW | 83 | 68 | 151 | 0.70 | 0.070 |
| Integrated | CC | 151 | 0 | 151 | 0.81 | 0.085 |
| _ | CSW | 83 | 68 | 151 | 1.02 | 0.100 |
| Compost | CC | 117 | 34 | 151 | 1.11 | 0.101 |
| | CSW | 49 | 102 | 151 | 1.17 | 0.104 |

[†] CC = continuous corn; CSW = corn-soy-wheat rotation.

since it requires shorter incubation times and less expensive instrumentation (Vahdat et al., 2010; Sherrod et al., 2012). Studies have shown that these two measures are highly related and that C mineralization is a sensitive indicator to recent changes in management (Franzluebbers et al., 2000; Haney et al., 2001; Franzluebbers and Stuedemann, 2008; Schomberg et al., 2009; Vahdat et al., 2010).

In this study, we examined how a suite of inexpensive labile soil C and N indicators respond to both short-term and long-term dynamics. We sampled soil six times throughout a season of corn to examine short-term dynamics and used a long-term cropping system trial to evaluate long-term changes due to historical management. The objectives of this study were: (i) to determine the impact that long-term management has had on a suite of simple soil tests related to labile organic C and N pools, (ii) to examine the short-term temporal dynamics of these organic C and N measures throughout the growing season, and (iii) to determine the capacity of these tests to predict corn agronomic performance defined as grain yield, total biomass, and total accumulated plant N.

MATERIALS AND METHODS

Site Description, Experimental Design, and Management

The study was conducted at the W.K. Kellogg Biological Station (KBS) in Hickory Corners, MI (42°24′ N, 85°24′ W, elevation 288 m). Mean annual precipitation is 890 mm and mean annual temperature is 9.7°C. The soils at this site are Kalamazoo series (fine-loamy, mixed, semiactive, mesic Typic Hapludalfs): http:// lter.kbs.msu.edu/about/site_description/index.php.

The long-term field trial (Living Field Laboratory) was established in 1993 to examine the effects of management, rotational diversity, and cover cropping on three field crops, corn, soybean [Glycine max (L.) Merr.], and wheat (Triticum aestivum L.) (Jones et al., 1998; Fortuna et al., 2003a; Sanchez et al., 2004; Snapp et al., 2010). The experimental design is a split–splitplot, randomized complete block design with four replications. Management is the main treatment, crop rotation is the subtreatment, and presence of cover-crops is the sub-subtreatment. From 1993 to 2005 the experiment was managed as a corn-cornsoy-wheat rotation with crimson clover (Trifolium incarnatum L.) interseeded into first-year corn, and annual ryegrass (Lolium multiflorum Lam.) interseeded into second-year corn. Soybean received no cover crop and red clover (*T. prantense* L.) was frost-seeded into the winter wheat. In 2006, some fundamental management changes were introduced to more closely reflect realistic cropping systems in the region and address additional

research questions. The main change was to reduce the rotation to a 3-yr corn-soy-wheat rotation, substituting crimson clover and annual ryegrass for the more popular and prolific cereal rye (Secale cereal L.) to be planted immediately after corn harvest. Another fundamental change was to normalize nutrient management so that all management treatments would receive the same estimated amount of N fertilizer despite the organic or inorganic form. This required adjusting N fertilization rates within each management after plots were credited for organic sources of N such as crop rotation, leguminous cover crop, and compost (see below). Additionally, cultural practices were normalized as much as possible across managements, including tillage practices (chisel plow), row cultivation for weed control, and herbicide applications and genetics (for non-organic plots).

Three management systems were examined in this study: Conventional (Conventional), Integrated Fertilizer (Integrated), and Integrated Compost (Compost). Conventional management represents typical farmer practices in the region with regards to soil management, fertilizer rates and application practices, and herbicide applications. Integrated management historically followed low-input practices, targeted applications of herbicide (one-third rate of commercial practice), reduced tillage, and detailed accounting of N inputs to minimize N fertilizer requirements. Since 2006, the management of Integrated plots has been identical to Conventional management. Compost management was historically identical to Integrated with the exception that all fertilization was provided with composted dairy manure. Since 2006, Compost management has received both compost and mineral N fertilizer (rates adjusted for organic N from compost). Composted manure was applied to all crops before planting except soybean [Glycine max (L.) Merr.] at a rate of $\sim 100 \text{ kg N ha}^{-1} (4 \text{ Mg ha}^{-1} \text{ dry weight basis; } 19-29 \text{ g N kg}^{-1},$ 250–380 g C kg⁻¹; Fortuna et al., 2003a).

Within these three management systems, two crop rotations were examined: (i) continuous corn with no cover crops, (ii) corn-soybean-wheat with cover crops. Every corn plot was fertilized with a targeted N rate of 151 kg ha⁻¹ with N credits given to rotation, red clover, and compost of 34 kg ha⁻¹, creating a range of fertilizer N rates from 49 to 151 kg ha⁻¹ for the various combinations of treatments in this study (Table 1). Fertilizer P and K was applied to Conventional and Integrated systems at a rate of 50 kg ha^{-1} of P_2O_5 and 84 kg ha^{-1} of K_2O on 5 Apr. 2011; whereas the Compost system did not require additional P and K (Tri-State Fertilizer Recommendation). On 6 May, glyphosate (0.46 kg a.i. ha⁻¹) was applied to red clover. All corn plots were chisel plowed followed by a soil finisher on 12 May. Pioneer corn

[‡] Corn was fertilized at a targeted rate of 151 kg N ha-1 with N credits of 34 kg N ha-1 given for crop rotation, red clover, and/or compost.

Table 2. Plant and soil sampling dates and corresponding plant development stages during the 2011 growing season.

| Sampling | Plant stage | Date (2011) | Measurements taken† |
|------------------------|-------------|-------------|--|
| Pre-plant | - | 15 May | Soil |
| Fifth leaf | V5 | 9 June | Soil, leaf chlorophyll |
| Tenth leaf | VI0 | 8 July | Soil, leaf chlorophyll |
| Anthesis | RI | 22 July | Soil, leaf chlorophyll, leaf area, and biomass |
| Milk stage | R3 | 16 August | Soil, leaf chlorophyll |
| Physiological maturity | R6 | 3 October | Soil, grain and whole plant biomass |

[†] Soil measurements include permanganate oxidizable carbon, C mineralization, N mineralization, nitrate, and ammonium.

hybrid PO413 was planted on 16 May at a rate of 69160 seeds ha $^{-1}$. Urea (46-0-0) was hand-applied as a starter fertilizer immediately following planting at a rate of 34 kg ha $^{-1}$. The pre-emergence herbicide, Lexar (S-metolachlor, mesotrione, and atrazine), was applied (3.31 kg total a.i.ha $^{-1}$) to corn on 16 May. Calcium ammonium nitrate (27-0-0) was hand-applied as a side-dress application on 21 June. A row cultivator was used on all plots to incorporate fertilizer N immediately following application.

Soil Sampling and Analyses

Soils were sampled in April 2008 before corn was planted for soil organic carbon (SOC) and total soil nitrogen (TSN). Soil was sampled again in 2011 in the corn phase of the rotation on six dates corresponding to key times in the corn lifecycle (Table 2). The full plow layer (0–25 cm) was sampled in accordance with previous studies in this long-term trial. Five cores (2 cm diam.) were collected between rows in each plot, composited, sieved to 6 mm, mixed until homogeneous. Soil moisture was determined gravimetrically. Soil organic C and TSN were determined on airdried soil by dry combustion on a Costech ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia, CA).

Inorganic Soil Nitrogen

Inorganic soil N was measured colorimetrically for nitrate (Miranda et al., 2001; Doane and Horwáth, 2003) and ammonium (Forster 1995). A 1 M potassium chloride extraction (40 mL per 10 g of fresh soil), which was shaken (240 rpm) for 1 h and centrifuged (2000 rpm) for 2 min. After filtration (2.5 μm, Whatman no. 42), extracts were frozen until analysis. Nitrogen mineralization (N-min) was determined following Drinkwater et al. (1996) by adding 10 g fresh soil and 10 mL deionized water to a 50 mL centrifuge tube, and purging the headspace with N₂ gas. Tubes were incubated at 37°C for 7 d and then 30 mL of 1.33 M potassium chloride was added to the tubes. Tubes were shaken, centrifuged, and extractant was filtered as described above. Nitrate and ammonium concentrations of soil extracts were determined with a SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA). Nitrogen mineralization potential was calculated as the difference between the incubated and initial ammonium.

Carbon Mineralization

One day carbon mineralization (C-min) was determined on rewetted soils following Franzluebbers et al. (2000) and Haney et al. (2001). The amount of water needed to bring soils to 50% water-filled pore space (WFPS) was determined gravimetrically before the incubations (Haney and Haney, 2010). Ten grams of air-dried soil were weighed into 100-mL beakers and placed inside a 237-mL canning jar. Deionized water was added to the soil, jars were capped tightly, and a zero time ${\rm CO}_2$ concentration

was determined immediately by sampling 0.5 mL of air from the headspace and injecting it into a LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE). The jars were incubated at 25°C in the dark for 24 h and a 1-d $\rm CO_2$ concentration was determined. Carbon mineralization was determined as the difference between zero time and 1-d $\rm CO_2$ concentration.

Permanganate Oxidizable Carbon

Permanganate oxidizable carbon was based on a Weil et al. (2003), but modified slightly as described by Culman et al. (2012). Briefly, 20 mL of 0.02 M KMnO $_4$ was added to a 50-mL centrifuge tube containing 2.5 g of air-dried soil. The tubes were shaken for exactly 2 min (240 rpm), allowed to settle for exactly 10 min, and 0.5 mL of the supernatant were transferred into a second 50-mL centrifuge tube and mixed with 49.5 mL of deionized water. Sample absorbance was read with a SpectraMax M5 spectrophotometer at 550 nm.

Plant Sampling and Analyses

Leaf chlorophyll content was determined at four stages of corn development to assess plant N status (Table 2). Chlorophyll content was measured indirectly by measuring leaf greenness with a Minolta SPAD-502 m (Konica Minolta, Ramsey, NJ) on the youngest, fully-collared leaf for 5th and 10th leaf samplings, and on the leaf above the primary ear for the anthesis (R1) and milk stage (R3) samplings. The average of 20 randomly selected leaves per plot was taken on unblemished areas halfway between the midrib and the edge of the leaf.

Leaf area was determined at anthesis by sampling whole leaves immediately above each primary ear from eight randomly sampled plants per plot. Leaf area was determined with a LI-COR LI-3100 leaf scanner and biomass was determined after oven drying at 60°C. At physiological maturity, eight whole plants per plot were randomly sampled (excluding previously sampled plants) to determine aboveground biomass and harvest index. Plants were separated into grain and remaining components and oven dried at 60°C. Corn grain yields were determined by hand harvesting all corn ears from the middle two rows in each plot (including grain from eight plant samples), and adjusting yields for moisture. Final total aboveground plant biomass (Mg ha⁻¹) was calculated by dividing final grain yield by harvest index. All biomass and grain yields are reported as oven-dried weights (0% moisture). Total tissue N was measured on anthesis leaves, harvest grain, and harvest vegetative samples. Tissues were ground to pass a 1-mm screen in a Christy-Turner Mill (Christy Turner Ltd., Ipswich, Suffolk, UK) and analyzed for total C and N using a Costech ECS 4010 CHNSO Analyzer.

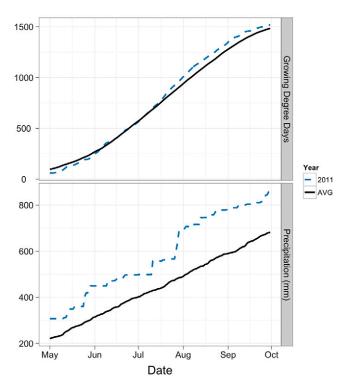


Fig. 1. Cumulative growing degree days and precipitation from May through October for the 2011 growing season (blue dashed line) and 24 yr average (black solid line) at the Kellogg Biological Station. Growing degree days were calculated by Baskerville-Emin (BE) method (Baskerville and Emin, 1969) with base = 10°C.

Statistical Analyses

Analysis of variance was performed on soil and plant data with the PROC MIXED procedure in SAS v.9 (SAS Institute, Cary, NC). Management history, and crop rotation were treated as fixed effects and block as a random effect with significant differences determined at $\alpha = 0.05$. For plant and soil variables measured multiple times throughout the growing season, we modeled sampling date as a repeated measure. Means were compared with an adjusted Tukey's pairwise means comparison procedure in PROC MIXED. The ability of measures to predict plant performance was assessed with correlation analyses and stepwise multiple linear regressions in R (R Core Team, 2012). Stepwise multiple linear regression was run with the function regsubsets() from the leaps package, which uses an exhaustive search (all possible combinations included) and ranks predictors in order of importance based on Mallows' C_p . The C_p also determines which model is the most

parsimonious (Mallows, 1973). All graphing was performed with the package *ggplot2* (Wickham, 2009) in R.

RESULTS AND DISCUSSION Weather

Overall, 2011 was characterized as a favorable growing season for Southwest Michigan with average temperatures and greater than normal precipitation (Fig. 1). In the spring, temperatures were lower than average, but by corn anthesis, 44 more growing degree days than the 24 yr average had accumulated. Precipitation in 2011 was greater than average at planting and this carried through anthesis (102 mm more than average) and throughout grain fill (172 mm more than average).

Soil and Plant Measurements

Soil sampled in 2008 showed minimal differences among total soil C or N pools across treatments (Table 1). Total SOC was marginally different between managements (F-statistic = 3.11, P = 0.094), but not different between crop rotations (F-statistic = 2.37, P = 0.158) nor was an interaction observed between management and rotation (F-statistic = 2.03, P = 0.187). Total soil N was unaffected by management (F-statistic = 0.08, P = 0.925), rotation (F-statistic = 0.49, P = 0.501) and the interaction of management and rotation (F-statistic = 1.20, P = 0.344).

In contrast to total soil C and N, labile measures of soil organic matter in 2011 showed large treatment differences (Table 3). In particular, crop rotation had a significant effect on all measured properties. Larger *F*-statistics with crop rotation relative to management indicate that rotation had a greater net effect than management on all soil and plant measures with the exception of POXC. Management history only affected POXC, C mineralization, and N mineralization (Table 3).

Labile Carbon Pools

Permanganate oxidizable C values show increases in POXC were driven by both management and rotational diversity (Fig. 2). Interestingly, differences in mean POXC values between Compost continuous corn and Compost corn—soy—wheat were minimal (management × rotation interaction, P=0.089), while differences between crop rotations in Integrated and Conventional management were more pronounced (Fig. 2). Overall, a more diverse crop rotation increased POXC values, but the relative effect of crop rotation on POXC was approximately half of that of management history (Table 3; F-statistic for Rotation = 64.8; F-statistic for Management = 130.3). Previous studies have reported POXC values in corn to increase due to crop diversity (Min et al., 2003; Jokela et

Table 3. Soil and plant F-statistics and significance from repeated measures ANOVA of all sampling dates (n = 144).†

| Source | POXC | C-min | N-min | NO ₃ -N | NH₄-N | Inorganic N | Chlorophyll |
|-----------------------|-----------|----------|----------|--------------------|----------|-------------|-------------|
| Management (M) | 130.3 *** | 22.0 *** | 8.6 ** | 2.3 | 5.0 | 3.2 | 2.4 |
| Rotation (R) | 64.8 *** | 58.2 *** | 59.9 *** | 17.7 ** | 32.7 *** | 6.4 * | 80.4 *** |
| $M \times R$ | 3.2 | 2.0 | 0.3 | 2.5 | 13.1 ** | 7.2 * | 2.9 |
| Date (D) | 28.3 *** | 15.0 *** | 13.7 *** | 87.2 *** | 9.3 *** | 55.5 *** | 98.2 *** |
| $M \times D$ | 0.4 | 1.5 | 0.8 | 1.7 | 2.5 * | 2.1 * | 1.1 |
| $R \times D$ | 0.5 | 3.1 * | 2.9 * | 6.5 *** | 3.3 * | 5.5 *** | 3.1 |
| $M \times R \times D$ | 1.7 | 1.1 | 0.7 | 0.5 | 2.8 ** | 1.3 | 1.4 |

^{*} Significance level: P < 0.05.

^{**} Significance level: P < 0.01.

^{***} Significance level: P < 0.001.

[†] POXC = permanganate oxidizable carbon; C-min = carbon mineralization; N-min = nitrogen mineralization; Inorganic N = (NO₃-N + NH₄-N).

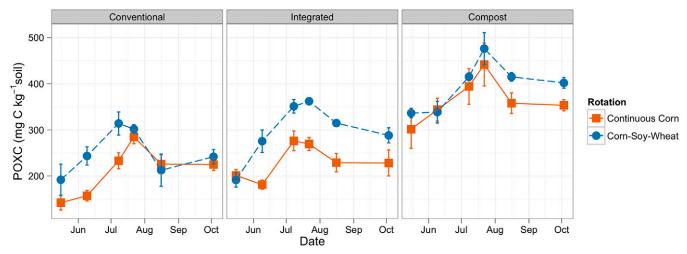


Fig. 2. Soil permanganate oxidizable carbon (POXC) values for continuous corn (red squares, solid line) and corn-soy-wheat rotations (blue circles, dashed line) across three management regimes. Error bars represent one standard error of the mean. Sampling dates correspond to key stages in corn development (Table 2; n = 24 for each sampling).

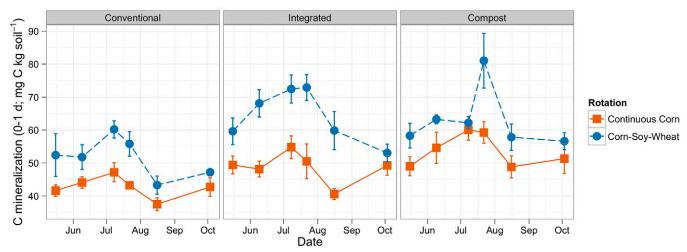


Fig. 3. Soil carbon mineralization (C-min) values for continuous corn (red squares, solid line) and corn-soy-wheat rotations (blue circles, dashed line) across three management regimes. Error bars represent one standard error of the mean (n = 24 for each sampling).

al., 2009; Spargo et al., 2011; Lucas and Weil, 2012) and applications of manure (Min et al., 2003; Mirsky et al., 2008), while some studies have reported no difference (Wienhold, 2005; Lewis et al., 2011). Mean POXC values roughly reflect the same trends of SOC values regarding management and crop rotation: rotations receiving compost had larger POXC and SOC values than conventional rotations (Table 1, Fig. 2). Likewise, corn—soy—wheat rotations had greater POXC and SOC values than continuous corn across all three managements. The agreement between soil POXC and SOC values is consistent with previous studies (Weil et al., 2003; Culman et al., 2012; Lucas and Weil 2012).

Soil C mineralization rates were strongly influenced by both crop rotation and management (Fig. 3) with rotation having nearly three times the effect as management (Table 3; *F*-statistic for Rotation = 58.2; *F*-statistic for Management = 22.0). Carbon mineralization shows a similar bell-shaped trend as POXC throughout the growing season, indicating the large influence of plant growth and/or soil temperature on both fractions of soil C. Similar bell-shaped patterns were reported in other labile C pools (i.e., particulate organic C and microbial biomass C) at the same site (Willson et al., 2001). However, differences exist

between C mineralization and POXC, most notably in response to Compost management. Additions of stabilized C had an overriding influence on POXC (Fig. 2), while C mineralization was influenced more by rotational diversity than management (Fig. 3, Table 3). A possible explanation for the difference is that C mineralization is more closely linked with crop residue quality than POXC. For example, in diverse crop rotations, the biochemical properties of leguminous residues with narrow C/N ratios may help alleviate N limitations, relative to stabilized C in compost with a wide C/N ratio (Fortuna et al., 2003a). Legumes often have diversified C substrate pools that are accessible to a wide-range of microbial taxa leading to increased rates of respiration (Spehn et al., 2000).

Permanganate oxidizable C and C mineralization represent conceptually different components of the soil: POXC is a chemically-extracted fraction of soil C and C mineralization is a laboratory incubation mediated by microbial biomass and activity. Wang et al. (2003) found a significant relationship (r=0.87) between C mineralization (7 d) and concentrated permanganate oxidation $(33.33 \text{ mM KMnO}_4)$. This study was the first to examine the relationship between POXC and C mineralization

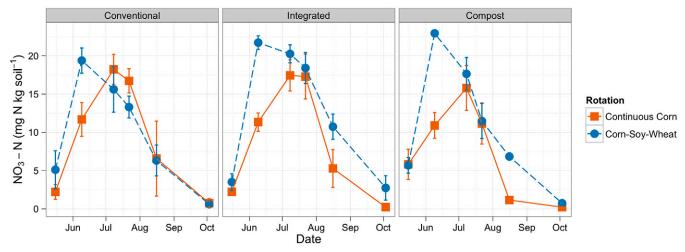


Fig. 4. Soil nitrate values for continuous corn (red squares, solid line) and corn-soy-wheat rotations (blue circles, dashed line) across three management regimes. Error bars represent one standard error of the mean (n = 24 for each sampling).

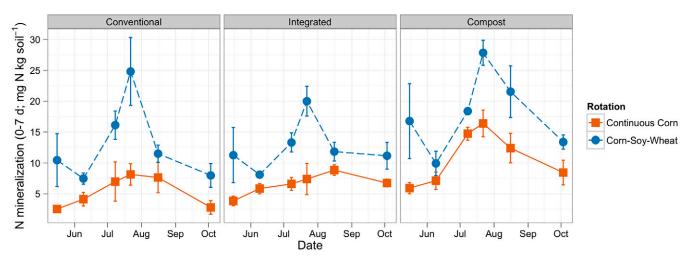


Fig. 5. Soil nitrogen mineralization potential (N-min) values for continuous corn (red squares, solid line) and corn-soy-wheat rotations (blue circles, dashed line) across three management regimes. Error bars represent one standard error of the mean (n = 24 for each sampling).

over short- and long-time intervals. Over all sampling dates in this study, C mineralization and POXC were related (r = 0.60), but the strength of the relationship suggests that the two measures respond differentially to C inputs (i.e., compost vs. crop rotational diversity).

Soil Nitrogen Pools

Soil nitrate status varied with rotation, but not with management history (Fig. 4, Table 3). Continuous corn inorganic nitrate was generally lower than corn-soy-wheat rotations, with the exception of a few samplings in Conventional plots (Fig. 4). For all three management systems, a temporal trend was also observed, as nitrate values in the corn-soy-wheat rotation peaked earlier (V5) than continuous corn (V10). The earlier peak in corn-soy-wheat rotations was likely due to a greater proportion of inorganic N coming from organic matter mineralization relative to continuous corn. Interestingly, even though inorganic N fertilization occurred immediately after V5 sampling (see Table 1 for rates), the next sampling (V10, third sampling) showed a decrease in soil nitrate for the corn-soy-wheat rotations, where continuous corn plots showed an increase in soil nitrate levels. The decrease in soil nitrate in the corn-soy-wheat rotation was likely the result of greater N uptake by more rapidly growing plants (see plant data below).

Management did not alter soil nitrate status, which suggests that crop access to N was comparable throughout the growing season, and that our N credits for organic N sources were appropriate. Total soil inorganic N (NO₃–N + NH₄–N) was primarily composed of NO₃–N with the percent of NO₃–N averaged over all sampling dates as follows: Conventional CC = 54.8%; Conventional CSW = 68.0%; Integrated CC = 59.4%; Integrated CSW = 74.7%; Compost CC = 62.4%; Compost CSW = 75.9%. Ammonium values were generally below 5 mg N kg soil $^{-1}$ in all but a few sampling time points in Conventional and Integrated management (data not shown).

Soil N mineralization was affected by both management history and crop rotation with rotation having nearly seven times the effect as management (Table 3, Fig. 5). For all three management systems, N mineralization was greater in the corn—soy—wheat rotation compared with continuous corn, especially during the V10 through R3 time period, coinciding with the linear growth phase and a period of peak plant N demand (Abendroth et al., 2011). Nitrogen mineralization was also notably greater in continuous corn in the Compost system, compared to continuous corn plots in the Conventional and Integrated systems. These results are consistent with earlier findings in the same experiment

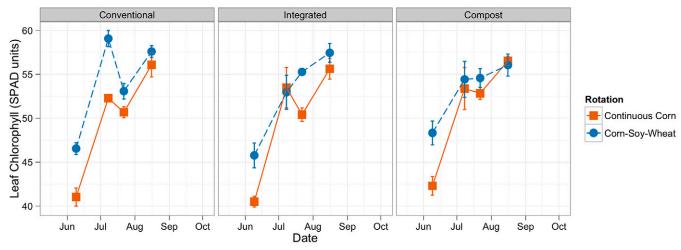


Fig. 6. Plant leaf chlorophyll values for continuous corn (red squares, solid line) and corn-soy-wheat rotations (blue circles, dashed line) across three management regimes. Error bars represent one standard error of the mean. Sampling dates correspond to key stages in corn development (Table 2; n = 24 for each sampling).

(Fortuna et al., 2003a; Sanchez et al., 2001) and other field trials (Gentry et al., 2001; Spargo et al., 2011). Our results demonstrate the long-term effect that continuous corn has on mineralizing labile organic matter (Sanchez et al., 2004) and suggests that the stabilized C addition of compost was vital for increasing the soil N supply in continuous corn.

Plant Responses to Management History and Crop Rotation

Plant leaf chlorophyll indicated temporal trends in soil N availability throughout the growing season. Overall, corn leaf chlorophyll was significantly greater in the corn-soy-wheat rotation compared with continuous corn (Fig. 6), but was not affected by management history (Table 3). Differences in chlorophyll content between crop rotations was greatest at V5 (first sampling) due to greater levels of soil nitrate (Fig. 4) from net mineralization of soil organic matter in the early spring in plots with a more diverse crop history. The next chlorophyll readings at V10 were much higher than at V5, reflecting the uptake of inorganic N fertilization applied to all plots (at different rates, Table 1) immediately after V5 sampling. Chlorophyll meter readings at anthesis showed differential responses to management and crop rotation: all continuous corn plots and the Conventional corn-soy-wheat rotation experienced a decrease in leaf chlorophyll. This suggests that plants were experiencing N stress during this time of peak growth rate and high N demand. In contrast the corn leaves in the corn-soy-wheat rotation in the Integrated and Compost systems experienced a minimal increase in chlorophyll at anthesis as these plants continued to accumulate N from a mineralizing pool of soil organic matter. At the next sampling (R3), leaf chlorophyll in all plots increased to their maximum N status, which was not influenced by management system (F-statistic = 0.14, P = 0.874) or rotational diversity (F-statistic = 1.28, P = 0.510).

At anthesis, the area of the leaf immediately above the primary ear was influenced by both rotation (F-statistic = 72.1, P < 0.001) and management (F-statistic = 4.26, P = 0.027). Leaf biomass was only affected by rotation (F-statistic = 52.9, P < 0.001), not management (F-statistic = 1.89, P = 0.190); however, the total N in the leaf was not affected by either rotation or management.

At physiological maturity (R6) harvested components of corn were affected mostly by crop rotation, with some responses due to management history. Corn grain yield, vegetative biomass and total N in vegetation were substantially greater in the cornsoy—wheat rotation than in continuous corn (Table 4). Harvest index was significantly less in the corn—soy—wheat rotation than in continuous corn due to greater vegetative growth in the more diverse systems (Table 4). Management history had an effect on vegetative biomass and harvest index, with Compost management having moderately higher vegetative biomass and lower harvest index than Conventional management (Table 4).

Sensitivity of Labile Soil Carbon and Nitrogen Measurements

Studies typically show increases in SOC after repeated applications of compost or manure (Ros et al., 2006; Reeve et al., 2012) and with reduced tillage practices (Grandy et al., 2006). Even though our compost application rate was relatively low (4 Mg compost ha⁻¹ yr⁻¹), we were surprised to find a lack of strong differences in SOC and TSN after 17 yr of management. This is likely a result of the diminished statistical power with a small sample size for SOC and TSN (one sampling, n = 24). On the contrary the labile soil C and N measures were sampled six times over the growing season (n = 144), since they are more temporally variable than SOC and TSN (Wander, 2004). In this same field trial 11 yr earlier in 2000, Sanchez et al. (2004) reported significant differences in SOC and TSN across management systems when all crop rotations were included, and these findings were confirmed in a 2008 sampling that included all treatments (Snapp et al., 2010). Therefore, the lack of differences observed with our subset of treatments likely resulted from a well-documented phenomenon: with small sample sizes the spatial heterogeneity of SOC and TSN is large enough to mask biologically meaningful differences in the field (Kravchenko and Robertson, 2011).

The F-statistics from ANOVA were also used to assess the sensitivity of measured soil and plant variables to experimental factors. Overall, measurements of labile C and N organic pools (POXC, C mineralization, and N mineralization) had larger F-statistics than inorganic N measures (NO $_3$ -N, NH $_4$ -N, and total inorganic N), indicating that labile organic pools were more

Table 4. Treatment means and F-statistics of corn component biomass and total N (n = 24).

| | | | Biomass | | | | Total N | |
|----------------|-----------|---------|--------------------|---------|---------------|-------|------------|---------|
| Management | Rotation‡ | Grain | V egetative | Total | Harvest index | Grain | Vegetative | Total |
| Conventional | CC | 9.10b | 6.97d | 16.07b | 0.57a | 123.9 | 33.4b | 157.3b |
| | CSW | 10.00ab | 8.73bc | 18.73ab | 0.53ab | 130.1 | 53.1ab | 183.3ab |
| Integrated | CC | 9.29b | 6.51 d | 15.79b | 0.59a | 118.8 | 30.9b | 149.7b |
| | CSW | II.61a | 10.25ab | 21.87a | 0.53ab | 136.6 | 78.2a | 214.7a |
| Compost | CC | 9.59ab | 8.12cd | 17.71b | 0.54a | 128.8 | 37.5b | 166.3b |
| | CSW | 9.85ab | 11.08a | 20.94a | 0.47b | 137.5 | 81.6a | 219.1a |
| Source | | | | | F-statistic | | | |
| Management (M) | | 2.6 | 6.2* | 3.9 | 5.8* | 1.7 | 2.5 | 1.5 |
| Rotation (R) | | 11.6** | 124.1*** | 77.8*** | 33.0*** | 1.3 | 66.5*** | 18.1*** |
| M × R | | 3.1 | 5.2* | 5.4* | 1.2 | 0.1 | 3.7 | 1.6 |

^{*} Significance level: P < 0.05.

Table 5. Correlation coefficients showing single soil and plant properties that best predict corn agronomic performance (grain yield, total aboveground biomass and total plant aboveground N) for the first three samplings (n = 24).†

| aboveground (4) for the materials amplings (n 24). | | | | | | | |
|--|-------------|---------------|---------|--|--|--|--|
| Stage/Measure† | Grain yield | Total biomass | Total N | | | | |
| SOC | 0.37 | 0.55 ** | 0.42 * | | | | |
| TSN | -0.2 I | -0.23 | -0.07 | | | | |
| Pre-plant | | | | | | | |
| POXC | 0.05 | 0.29 | 0.43* | | | | |
| C mineralization | 0.61** | 0.61*** | 0.53** | | | | |
| NO ₃ -N | 0.36 | 0.35 | 0.22 | | | | |
| N mineralization | 0.39 | 0.59** | 0.34 | | | | |
| V5 | | | | | | | |
| POXC | 0.25 | 0.51** | 0.36 | | | | |
| C mineralization | 0.64*** | 0.74*** | 0.40* | | | | |
| NO ₃ -N | 0.41* | 0.18 | 0.27 | | | | |
| N mineralization | 0.21 | 0.55** | 0.44* | | | | |
| Chlorophyll | 0.08 | 0.09 | 0.26 | | | | |
| VI0 | | | | | | | |
| POXC | 0.35 | 0.56** | 0.47* | | | | |
| C mineralization | 0.61*** | 0.78*** | 0.57** | | | | |
| NO ₃ -N | 0.57** | 0.78*** | 0.79*** | | | | |
| N mineralization | 0.53** | 0.60** | 0.49* | | | | |
| Chlorophyll | 0.23 | 0.57** | 0.53** | | | | |

^{*} Significance level: P < 0.05.

sensitive measures to changes due to management history and crop rotation than inorganic N (Table 3). The F-statistics also reveal that NO_3 –N and leaf chlorophyll were the most temporally variable measurements, followed by POXC, C mineralization, and N mineralization. Soil inorganic N was previously shown to exhibit high seasonal variability in this study (Fortuna et al., 2003b). Finally, the most sensitive indicator of both management history and crop rotation was POXC, followed by C mineralization and N mineralization. These measurements of labile organic matter have been previously shown to be sensitive indicators to changes in the soil ecosystem (Franzluebbers et al., 2000; Schomberg et al., 2009; Culman et al., 2012), but these results provide further insight by quantifying their relative sensitivity to one another, to management differences, and to seasonal variability.

Ability of Measures to Predict Plant Agronomic Performance

A long-term goal of sustainable agriculture research is to develop a labile soil organic matter test, or suite of tests, that are able to predict agronomic performance, and in turn, provide fertilization recommendations. We were interested in assessing the ability of the simple labile organic matter measures to relate to crop performance, specifically, grain yield, total aboveground biomass and total aboveground N. We first ran simple bivariate correlations to determine if any of the measured variables were individually able to predict plant performance at harvest. We then ran stepwise multiple regressions to determine the ability of a combination of variables to determine corn agronomic performance.

Correlation analyses from the first three samplings revealed that many of the measured plant and soil properties were significantly and positively related to grain yield, total biomass, and total N (Table 5). As the field season progressed, the strength of many relationships grew stronger than in the beginning of the season (e.g., V10 vs. pre-plant). Carbon mineralization was more strongly related to yield and total biomass than any other indicator in the first three samplings. Nitrogen mineralization ranked second in consistency behind C mineralization. Soil nitrate was weakly related to plant performance in the first two samplings, but was strongly related to plant performance in the third sampling. Collectively, these relationships reveal the sensitivity of the biologically-mediated pools of C and N in predicting plant performance, as has been previously shown with C mineralization (Haney et al., 2001; Sanchez et al., 2004), N mineralization (Spargo et al., 2011), and POXC (Weil et al., 2003; Lucas and Weil, 2012).

Stepwise regression analyses selected the combination of indicators that were most parsimonious for predicting corn agronomic performance (Table 6). Since using automated model selection can be problematic for a variety of reasons (Whittingham et al., 2006), this analysis is intended to explore general patterns rather than assume a specific relationship for a response at a specific sampling. The predictors in the final model in Table 6 are listed in order of greatest to least importance. Considerable improvements in model *F*-statistics were made by selecting a subset of variables. Overall, trends are consistent with correlation analyses that C mineralization and N mineralization are the best predictors of corn agronomic performance early in the season before crop

^{**} Significance level: P < 0.01.

^{***} Significance level: P < 0.001.

 $[\]dagger$ Different letters in the same column represent significantly different treatment means.

[‡] CC = continuous corn; CSW = corn-soy-wheat rotation.

^{**} Significance level: P < 0.01.

^{***} Significance level: P < 0.001.

[†] SOC = soil organic carbon; TSN = total soil nitrogen; POXC = permanganate oxidizable carbon; SOC and TSN only measured one time, not at each stage.

Table 6. Stepwise multiple linear regression models and F-statistics showing which set of labile C and N measures best predict corn agronomic performance (grain yield, total aboveground biomass, total plant aboveground N)†.

| Response variable | Predictors in full model | F-statistic | Predictors in final model | F-statistic | |
|-------------------|---|-------------|---|-------------|--|
| Pre-plant | | | | | |
| Grain yield | C-min, POXC, N-min, NO ₃ -N | 3.73 | C-min | 12.84 | |
| Total biomass | C-min, POXC, N-min, NO ₃ -N | 4.65 | C-min, N-min | 9.89 | |
| Total N | C-min, POXC, N-min, NO ₃ -N | 2.59 | C-min | 8.78 | |
| V5 | • | | | | |
| Grain yield | C-min, POXC, N-min, NO ₃ -N, Chlorophyll | 6.43 | C-min, NO ₃ –N, Chlorophyll, N-min | 7.68 | |
| Total biomass | C-min, POXC, N-min, NO ₃ -N, Chlorophyll | 16.53 | NO ₃ –N, C-min | 40.51 | |
| Total N | C-min, POXC, N-min, NO ₃ -N, Chlorophyll | 8.09 | NO ₃ -N, POXC | 22.68 | |
| VI0 | · | | • | | |
| Grain yield | C-min, POXC, N-min, NO ₃ -N, Chlorophyll | 5.48 | C-min, NO ₃ –N | 14.64 | |
| Total biomass | C-min, POXC, N-min, NO ₃ -N, Chlorophyll | 6.64 | C-min, N-min, NO ₃ –N | 11.83 | |
| Total N | C-min, POXC, N-min, NO ₃ -N, Chlorophyll | 2.07 | N-min, NO ₃ –N | 5.05 | |

[†] POXC = permanganate oxidizable carbon; C-min = carbon mineralization; N-min = nitrogen mineralization.

planting and that other variables, particularly soil nitrate, become more predictive as the growing season progresses (V5 and V10).

Soil N supply indicators (N mineralization, nitrate, and leaf chlorophyll) had a predictive presence in the regression models in the V5 and V10 samplings that reflects the highly N responsive traits associated with corn (Fox et al., 1989; Binford et al., 1992). However, C mineralization played a surprisingly large role in predicting corn performance, relative to N supply indicators, as it was the most important predictor in six out of the nine total models. This suggests that C mineralization may be a more sensitive predictor of yields and agronomic performance in corn, than the two most commonly employed methods, PNST and leaf chlorophyll, especially in the early stages of the growing season. Additional studies are needed to address this question, but our results are consistent with Schomberg et al. (2009), who found that C mineralization (3 d after rewetting) was the best predictor of potentially mineralizable N. Additionally, other studies have shown relationships between C mineralization and both N mineralization and plant performance (Haney et al., 2001; Vahdat et al., 2010; Sherrod et al., 2012).

CONCLUSIONS

Our results indicate the measured labile soil organic matter indicators (POXC, C mineralization, and N mineralization) were able to reflect both short- and long-term dynamics in corn-based cropping systems in the upper Midwest. Overall, POXC was the most sensitive indicator of both management and crop rotational diversity, and C and N mineralization were more sensitive to treatment differences than inorganic N. Our results suggest that POXC and C mineralization are differentially influenced by inputs, as POXC was more influenced by stabilized C inputs and C-mineralization by greater substrate diversity from crop rotations.

Carbon mineralization was a better predictor of corn agronomic performance than any other measure, both as a single indicator, and in combination with other indicators. Carbon mineralization outperformed two currently recommended methods for measuring early-season corn N status, PSNT, and leaf chlorophyll content, demonstrating the potential of C mineralization in determining corn agronomic performance.

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