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Compost Legacy Down-Regulates Biological Nitrogen Fixation in a Long-Term Field Experiment

Sieg Snapp,* Brook Wilke, Lowell E. Gentry, and Danielle Zoellner

ABSTRACT

Biological nitrogen fixation (BNF) is a fundamental process relied on in agriculture, yet few field studies have examined regulation through soil inorganic N feedbacks or considered seasonal effects. In a Michigan long-term field experiment we examined soil labile C and N pools and impact on BNF in two species, over multiple years. The ^{15}N natural abundance method was used to quantify BNF, with nodulated and non-nodulated soybean [*Glycine max* (L.) Merr.] isolines and red clover (*Trifolium pratense* L.). Soil organic C status of plots was consistent with a gradient established through historical inputs: 0.8% (fertilizer management) to 0.9% (+ cover crop) to 1.2% (+ compost + cover crop). The fraction of nitrogen derived from atmosphere (fNdfa) in red clover and soybean over 3 yr was 61 to 58% with compost management, and 71 to 72% with fertilizer. This represented a downregulation of 19% (red clover) and 15% (soybean) in compost and cover crop managed plots, relative to inorganic fertilizer. In addition to management effects, we found that weather markedly influenced the total amount of N fixation over the 3 yr of the study. A mesic season supported vigorous soybean growth and 195 kg N ha^{-1} BNF, compared to 95 and 90 kg N ha^{-1} BNF in a dry and an excessively wet season. This study found that compost-based management increased pools of labile C and N that internally downregulated BNF, while enhancing soybean yield compared to conventional management.

Core Ideas

- Compost-based management enhanced soil organic matter in a Michigan Alfisol.
- Nitrogen fixation rates in soybean and red clover varied with management history.
- Weather markedly influenced the quantity of N fixed in three crop systems.
- Soil labile pool influence on N fixation can act as an internal regulator.

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Croplands dominate one-third of ice-free land, acting as both a source and sink for global N cycles (Galloway and Cowling, 2002). Management effects on BNF and feedback regulation by soil properties have been proposed as mechanisms to help support sustainable food production system, through regulation of excessive reactive N (Pearson, 2007). Yet there are few field-based studies that address external and internal regulation of N within row crop agriculture, and almost no long-term studies that include observations over multiple seasons. These processes need to be understood to tighten N cycling, and support use of management options that are environmentally friendly (Drinkwater and Snapp, 2007).

A comprehensive field survey of soybean in Argentina highlighted the variation in BNF associated with this important N-fixing crop (Collino et al., 2015). Soil properties were shown to play an important role in regulation of soybean BNF, as well as air temperature in high potential soybean production areas. In addition to such scoping studies, long-term field experimentation is required to understand management legacy effects on crop BNF. Retention of N through timing, inhibitors, and precision placement of fertilizer has been a primary focus in agricultural nutrient management to date. Despite the substantial role that BNF plays in agriculture, and the interest in expanding this role, there have been few multi-year, field based studies that examine management or weather effects on BNF.

Alternative crop management that builds labile organic matter pools, and also relies on organic N sources, have been proposed as means to reduce losses of reactive N (Bhowmik et al., 2016; Drinkwater and Snapp 2007). Specific mechanisms that could provide internal regulation of BNF include the role of inorganic N feedbacks, which have been shown to control the rhizobium–legume symbiosis which in turn regulate N fixation activity (Streeter and Wong, 1988; Arrese-Igor et al., 1997; Schipanski and Drinkwater, 2012). Inorganic N has been shown to downregulate N fixation in a controlled environment (Arrese-Igor et al., 1997) and in pasture-based studies (Burchill et al., 2014), but

S. Snapp, Dep. of Plant Soil and Microbial Science, Michigan State Univ., 1066 Bogue St., East Lansing, MI 48824; B. Wilke and D. Zoellner, Kellogg Biological Station, Michigan State Univ. 10461 N 40th St., Hickory Corners, MI 49060; L.E. Gentry, Dep. of Natural Resources and Environmental Sciences, Univ. of Illinois-Urbana-Champaign, W503 Turner Hall, 1102 S. Goodwin Ave. MC-047, Urbana, IL 61801. Received 12 Mar. 2017. Accepted 21 May 2017.
*Corresponding author (snapp@msu.edu).

Abbreviations: BNF, biological nitrogen fixation; CO−, conventional; fNdfa, fraction of nitrogen derived from atmosphere; IC+, integrated compost with cover crop; IF−, integrated fertilizer; IF+, integrated fertilizer with cover crop; NMP, nitrogen mineralization potential; POXC, permanganate oxidizable carbon.

the extent to which historical soil management influences BNF in row crops remains little studied. An on-farm study that evaluated a gradient of management practices that varied in reliance on organic N amendments found evidence for downregulation of BNF in fields with enhanced soil inorganic N and other altered edaphic properties (Schipanski and Drinkwater 2012). However, a field experiment using ^{15}N natural abundance and dilution techniques demonstrated the opposite effect: elevated soybean BNF rates under organic and compost-based management were found with enhanced soil organic N pools, relative to N-fertilizer management (Oberson et al., 2007). Neither of these studies were performed over multiple years, and seasonal effects in rain-fed production systems are expected to be important as soil moisture has been shown to strongly regulate BNF in many legumes (Purcell et al., 2004).

To understand internal regulation mechanisms in a field environment we used a long-term row-crop experiment to monitor labile N and C pools, quantify BNF, and assess crop response to historic N sources. Our focus crop was soybean, as a major BNF source in field crop systems around the globe (Herridge et al., 2008; Collino et al., 2015). We also evaluated BNF in red clover, which is one of the more widely grown cover crops in North American field crop production (Snapp et al., 2005). Our study was conducted over three seasons, which encompassed a broad range of climatic conditions. The long-term trial "Living Field Laboratory" at the Kellogg Biological Station provided an opportunity to evaluate legacy effects of four management systems, where N sources ranged from inorganic to organic. Our hypothesis was that internal regulation of N would be improved in management systems that historically relied on organic N sources (e.g., compost and cover crops) when compared to management that relied solely on soluble fertilizer, as BNF would be downregulated by N mineralization as soil labile pools accrued.

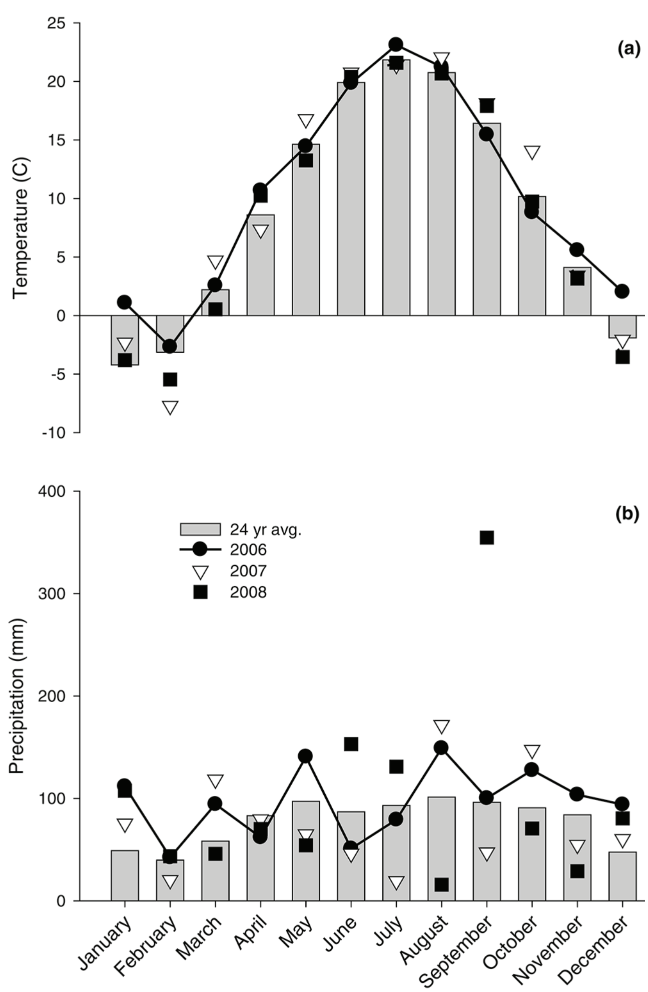

Fig. 1. Mean monthly temperature and monthly precipitation presented for 2006 to 2008 at the Living Field Lab long-term experiment. This trial is located at the W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI.

MATERIALS AND METHODS

Study Site

This experiment was conducted at the Living Field Laboratory (LFL) long-term field crop ecology experiment established in 1993 at the W.K Kellogg Biological Station of Michigan State University, located in Southwest Michigan (Sanchez et al., 2004; Culman et al., 2013). The area receives approximately 90 cm of precipitation annually, with approximately half as snow. Monthly mean precipitation and temperature for the study years 2006 to 2008 are illustrated in Fig. 1. The site is located on a mixture of Kalamazoo and Oshtemo sandy loam soils (both Typic Hapludalfs), a detailed site description can be accessed at http://lter.kbs.msu.edu/data/LTER.

The 30-yr average rainfall during the growing season (1 April through 30 September) at this site is 532 mm, similar to 2006 seasonal precipitation which totaled 582 mm and was well distributed (Fig. 1). However, in 2007, the growing season precipitation was only 429 mm with less than 50 mm over 6 wk in mid-summer (during June and July), which drought-stressed plants. In contrast, following an extremely dry August, excess rainfall was the challenge in 2008 (780 mm), where half of the annual rainfall occurred during September. This interfered with crop maturation and harvest (USDA-NASS, 2013).

Experimental Design

The experimental design is four randomized complete blocks, with main plots for management systems and split plots for crop sequence (Sanchez et al., 2004). The three rotational crops (corn [*Zea mays* L.], soybean, and wheat [*Triticum aestivum* L.]) are present within each main plot during each year. For this study we focused primarily on soybean and red clover, which was seeded as a cover crop into wheat during the month of March each year. Individual plots were 9.1 by 20.0 m, which accommodated 12 rows spaced 0.76 m apart for corn and soybean, and 48 rows 0.19 m apart for wheat. A factorial split-split plot of winter cover crop vs. winter fallow allows us to assess the effect of a management history of cover crop, for a final plot size of 4.5 by 10 m (Gentry et al., 2013).

Four management systems are the focus of this study: conventional (CO–), integrated fertilizer (IF–), integrated fertilizer with cover crop (IF+), and integrated compost with cover crop (IC+). Prior to 2006, the CO system used Michigan State University Extension (MSU Extension) recommended fertilizer rates, the IF system used an integrated approach for nutrient and weed management and the IC system an integrated compost-based approach, as described by Sanchez et al. (2004). Integrated management consisted of environmentally friendly practices of adjusting N fertilizer approximately one-third downward, based on pre-side dress soil inorganic N monitoring, and a one-third reduction of herbicide based on banded application. The IF+ system included two cover crops: a frost-seeded red clover in wheat sequenced before corn and a rye cereal cover crop between the soybean and wheat crop (historically an annual ryegrass–crimson clover mixture was used which was replaced with rye due to more reliable establishment, see Sanchez et al., 2004). The IC+ system was historically managed with applications of dairy compost before the cereal phases of the rotation and included winter cover crops as for IF+.

Subsequent to 2006, all systems used balanced N fertilizer rates applied to cereals: identical for CO– and IF– and adjusted downward for IF+ and IC+ based on a N credit system to take into account a N credit for the presence of a red clover cover crop, and historical compost application. Soybean was managed identically with no fertilizer or compost applied all systems during the 2006 to 2008 study period to evaluate historic management effects (and similarly no compost was applied to corn or wheat in rotation sequences over these years). Soybean BNF measurements were taken from 2006 to 2008 in IF–, IF+, and IC+. Red clover BNF measurements were taken during 2007 and 2008 in CO–, IF+, and IC+ plots (where red clover was grown for the first time in 2007 in the historically non-cover crop managed CO– plots). We sowed red clover in CO–, which were historically cover crop plots (Wilke, 2010). Thus, CO– was included in the study for the red clover comparisons only, based on its similar management history to IF–, and the lack of a red clover presence in IF–.

Management

This study was conducted within a corn–soybean–wheat rotation where wheat was planted in early fall directly after soybean harvest and seed bed preparation (soil finisher/field cultivator). Red clover was established as a relay intercrop in wheat on 20 Mar. 2007 and 2008, at a rate of 20 kg of seed ha^{-1}. Soybean was planted on 25 May 2006, 30 May 2007, and 22 May 2008, using cultivar Pioneer 92M72 in 2006 and 2007, and cultivar Pioneer 92m61 in 2008, seeded at a density of 150,000 plants ha^{-1}. To determine N fixation for soybean, nodulating and non-nodulating isolines of Williams 82 were planted in microplots (2 by 2.5 m) within each soybean plot, as described below.

Following MSU recommended practice, in all plots weeds were controlled with glyphosate[*N*-(phosphonomethyl)glycine] (0.3–0.4 kg a.i. ha^{-1}) in soybean applied in early May and *S*-metolachlor at 0.5 kg ha^{-1} a.i. and bromoxynil at 0.1 kg ha^{-1} a.i. applied in corn, depending on weed population density (cultivation controlled weeds in some cases). Weed control was performed by hand-hoe in the subplots of Williams 82 soybean (which were not treated with herbicide). As described in Culman et al. (2013), N fertilizer was applied in CO- and IF- systems to corn at planting (liquid fertilizer 25 kg ha^{-1} N), and side-dressed with ammonium nitrate at 75 kg N ha^{-1}. In IF+ and IC+ systems side-dressed ammonium nitrate treatment was adjusted downward to 58 and 24 kg N ha^{-1}, respectively. Nitrogen fertilizer was applied to wheat in March as urea 70 kg N ha^{-1} in the CO and IF systems and 47 kg N ha^{-1} in the IC system. Historically in the IC treatment dairy compost was applied at a rate of approximately 100 kg ha^{-1} of total N (C/N ratio ranged from 11:1–13:1) annually, approximately 1.8 Mg compost ha^{-1} annually to cereals in the rotation (Sanchez et al., 2004). During the study we suspended application of composted manure to investigate legacy effects.

Soil Measurements

Soil properties measured in 2008 for the 0- to 20-cm depth are presented in Table 1. A composite (eight subsamples) soil sample was collected for each plot, and analyses conducted as described previously (Snapp et al., 2010). Briefly, the hydrometer method (LTER, 2008) was used to measure soil texture, soils were ground to pass a 1-mm sieve, then extracted (Mehlich III) to evaluate soil chemical properties colormetrically. Subsamples were used to measure soil C and total N using a Carlo Erba NA1500 SeriesII Combustion Analyzer, and to measure labile C using the permanganate oxidizable carbon (POXC) method for colormetric analysis of soil extracted with 0.02 mol L^{-1} $KMnO_4$ (Weil et al., 2003, Culman et al., 2012). Soil texture varied from loam to sandy loam, Soil Mehlich III extractable inorganic P 29 ± 2 mg P kg^{-1} soil, K 80 ± 4 mg K kg^{-1}, and Ca 1130 ± 30 mg Ca kg^{-1} (Table 1). Soil C, total N, and POXC were all influenced by management system, with a gradient of SOC from 0.7% C under conventional N fertilizer management and 1.2% under compost management.

In 2006, 2007, and 2008 soil samples (0–25-cm depth) were collected to monitor inorganic N and nitrogen mineralization

Table 1. Mean (± 1 SE) of measured soil properties for the year 2008 in each management system examined in the Living Field Lab long-term experiment established in 1993 at the Kellogg Biological Station, Michigan State University, Hickory Corners.

Managment systems†	SOC‡	TSN	POXC	P	K	Mg	Ca	pH	CEC cmol
	%		mg kg^{-1} soil	mg kg^{-1}					kg^{-1} soil
CO–	0.72 (0.03)a§	0.07 (0.005)a	261 (12)a	24.5 (2.0)a	77.0 (2.3)	131 (6)a	1056 (51)a	7.3 (0.08)ab	6.6 (0.3)a
IF–	0.88 (0.05)ab	0.09 (0.002)b	303 (9)ab	24.1 (1.6)a	75.7 (3.3)	127 (3)a	1046 (32)a	7.2 (0.05)a	6.5 (0.2)a
IF+	0.94 (0.04)b	0.09 (0.003)b	360 (26)b	24.2 (1.7)a	78.8 (4.2)	134 (6)a	1033 (21)a	7.1 (0.06)ab	6.5 (0.1)a
IC+	1.19 (0.04)c	0.11 (0.001)c	477 (15)c	37.8 (2.6)b	84.8 (2.9)	156 (4)b	1296 (25)b	7.4 (0.03)b	8.0 (0.1)b
p value	<0.001	<0.001	<0.001	<0.001	ns¶	<0.001	<0.001	<0.01	<0.001

† CO– = Conventional no cover crop; IF– = Integrated fertilizer no cover crop; IF+ = Integrated fertilizer + cover crop; IC+ = Integrated compost + cover crop.

‡ SOC = soil organic carbon; TSN = total soil nitrogen; POXC = permanganate oxidizable carbon; Inorganic N = NH_4 + NO_3; NMP = nitrogen mineralization potential; CEC = cation exchange capacity.

§ Different letters within a column indicate significant differences among treatments using Tukey's HSD (p < 0.05) for the 2008 soil sampling.

¶ ns, not significant.

potential (NMP) mid-April, and June of each year. Eight subsamples were randomly collected and composited to represent a plot, sieved to pass 6 mm, and soil nitrate N was determined on fresh soil samples as described by McSwiney et al. (2010). In brief, we used 1 M KCl extraction, decanted into scintillation vials, froze and then analyzed using a SmartChem 140 (Westco Scientific Instruments, Inc., Brookfield, CT) discrete analyzer based on Cd reduction (USEPA, 1993). A subsample was dried at 105°C overnight to determine soil moisture content. Nitrogen mineralization potential was determined using an aerobic 30-d incubation of rewetted soils according to Beedy et al. (2010), in quadruplicate samples incubated at 25°C. Water was added to 50% of field capacity in containers that allowed atmospheric exchange (parafilm) and returned to 50% of field capacity, after being measured gravimetrically, on a weekly basis. After 30 d of incubation, each sample was extracted and inorganic N determined as described.

Plant Measurements

Soybean grain yield was determined in mid-October each year. Two yield rows were combine harvested per plot ($30.4\ m^2\ plot^{-1}$). Grain was weighed fresh and dry weight was determined, adjusting for moisture content using a DICKEY-john moisture meter (Churchill Industries, Minneapolis, MN).

The ^{15}N natural abundance method was used to provide an integrated measure of biological N fixation in soybean and red clover (Oberson et al., 2007). Non-nod soybean isolines (cultivar Williams) provided a non-fixing N reference value and were established in microplots (2 by 2.5 m) in each field plot (12 in all, four blocks × three management systems IF–, IF+, IC+). Wheat provided the non-fixing N reference value for red clover, which was established in microplots on 20 Mar. 2007 and 2008, in a standing wheat crop in each plot (12 in all, four blocks × three management systems, CO, IF+, IC+). The equation used to calculate the fNdfa follows:

$$\text{fNdfa} = (\delta^{15}N_{ref} - \delta^{15}N_{fix})/(\delta^{15}N_{ref} - \delta^{15}N_{b})$$

where "ref" are non-fixing and "fix" are nitrogen-fixing plants grown under the same conditions, and "b" is the fixing plant grown with atmospheric N_2 as the sole external nitrogen source (Oberson et al., 2007). To calculate the *B* value, we used was the most negative value detected, which in soybean was –1.43‰, and in red clover was –1.84‰. This is similar to earlier reported values for soybean (–1.2‰) (Oberson et al., 2007).

Aboveground biomass of soybean was measured using destructive sampling of two randomly located quadrats $0.5\ m^2$ harvested per plot 13 Sept. 2006, 12 Sept. 2007, and 23 Sept. 2008. This was at reproductive maturity stage R6, where tissue was separated into pod (including grain) and vegetative (leaf + stem) fractions and dried at 60°C until no change in weight. Red clover biomass was harvested in $0.25\ m^2$ quadrats on 3 May 2007 and 6 May 2008, and the remaining cover crop was killed and incorporated prior to planting corn. Tissue samples for soybean and red clover were ground to pass a 1 mm screen in a lab mill (Christy-Turner, Ipswich, Suffolk, UK) and plant tissue N content was determined using the combustion method at the Univ. of California Davis Analytical Isotope Lab (AOAC, 2006).

Statistical Analysis

We used R for all statistical analyses (R Development Core Team, 2015). Initially, "Block" was included in all ANOVA analyses as a random factor, but it was ultimately dropped when we found that it was not significant, nor did exclusion change the outcome for any of the analyses. To illustrate overall soil nutrient status in a single year, soil measurements taken in 2008 were examined using a single-factor ANOVA where Management (CO–, IF–, IF+, IC+) was the only independent variable. A single-factor ANOVA was also used to demonstrate the overall influence of Year (2006, 2007, 2008) on aboveground N status for the three plant types examined here (nodulating soybean, non-nodulating soybean, red clover). For inorganic N, NMP, soybean yield, nodulating soybean fNdfa, and red clover fNdfa, we employed a two-factor ANOVA model for Management and Year, as well as examining whether there was a Managment × Year interaction. We used Levene's test to ensure homogeneity of error variances. Tukey's HSD post-hoc test was used to compare means when we detected an overall effect using ANOVA. Results were considered significant when $p \leq 0.05$.

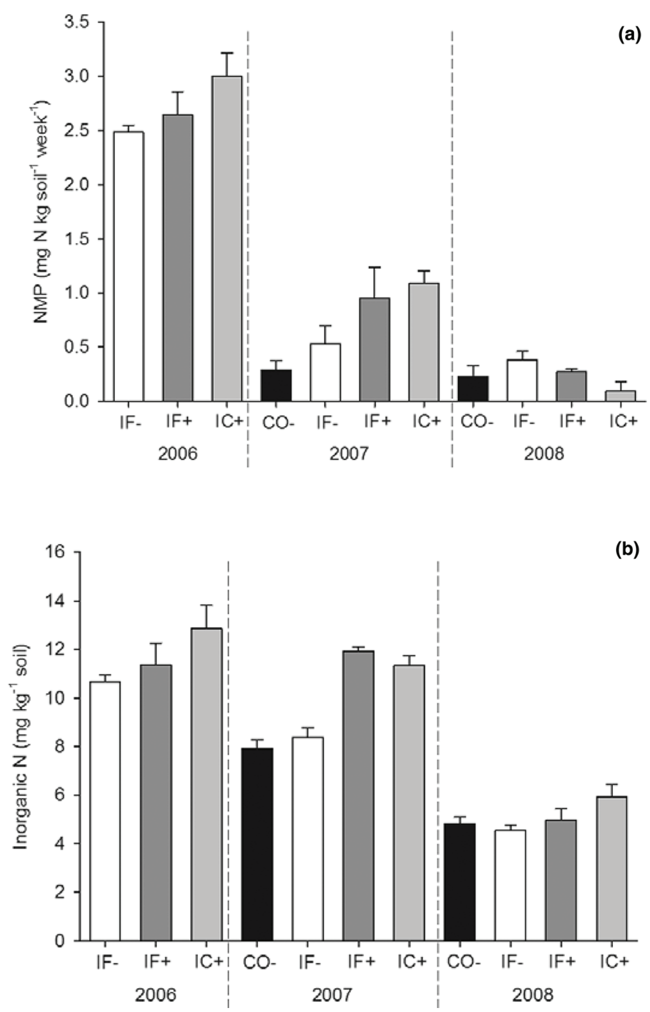

Fig. 2. (a) Nitrogen mineralization potential (NMP) and (b) inorganic N for soils in four different management regimes (CO– = conventional, no cover crop; IF– = integrated fertilizer, no cover crop; IF+ = integrated fertilizer, plus cover crop; IC+ = integrated compost, plus cover crop) over the 3-yr study period in the Living Field Lab long-term experiment at Kellogg Biological Station, Michigan State University, Hickory Corners, MI. Bars represent standard errors.

RESULTS

Soil Carbon and Nitrogen Status

Soil properties measured in 2008 reflected management history. The system that incorporated dairy compost, IC+, had the highest values for organic soil C, POXC active C, total soil N, P, Ca, and cation exchange capacity (Table 1). Relative to the fertilizer intensive IF– treatment, the IC+ system had soil organic C that was 35% higher, POXC that was 41% higher, and total soil N that was 22% higher ($p < 0.001$).

Soil inorganic N was temporally dynamic (Fig. 2), and influenced by both Year ($p < 0.001$) and Management ($p < 0.001$). In addition a Year × Management interaction was observed ($p < 0.05$). Overall, soil inorganic N was consistently lower in IF– than with historical compost (IC+; Fig. 2). For NMP both Year ($p < 0.001$) and Management ($p < 0.001$) were significant along with the Year × Management interaction ($p < 0.05$).

Crop Productivity

This field experiment was conducted over a time span that encompassed a full spectrum of precipitation patterns, from optimal for crop growth (2006), to dry during vegetative development (2007), to dry during grain fill with late excess moisture (2008). Not surprisingly, seasonal effects on grain production were marked ($p < 0.001$ in all crops). In the two-factor ANOVA testing Year × Management effects for soybean, both Year ($p < 0.001$) and Management ($p = 0.02$) were significant, but there was no interaction of Year × Management ($p = 0.54$). The best soybean yields were observed in 2006 (average ± SD: 2010 kg ha^{-1} ± 190), in 2007 yields were lower (1650 kg ha^{-1} ± 170) and in 2008 yields were the lowest (1270 kg ha^{-1} ± 140). Management history also influenced grain yield in soybean (Fig. 3): lowest in the conventionally fertilized, non-cover crop IF– system (average over the three seasons = 1470 kg ha^{-1} ± 180) and equivalent in IF+ and IC+ (1730 kg ha^{-1} ± 220).

Biological Nitrogen Fixation

The proportion of tissue N that was derived from BNF was measured each year, at soybean reproductive maturity (Fig. 4). In the two-factor ANOVA model testing Year and Management effects, only Management ($p = 0.03$) was significant, neither Year ($p = 0.13$) nor the Year × Management interaction ($p = 0.62$) were significant. Over the 3 yr, soybean BNF-derived fraction in vegetation was 55% in the IC+ system, lower than the 67 and 68% BNF observed in IF– and IF+ (Fig. 4; means comparison HSD $p = 0.06$ and 0.02, respectively). Numerical if not significant differences were observed in the same direction, with lower proportion of BNF- derived N observed in the IC+ system for soybean pod+seed tissues relative to IF (Table 2; $p = 0.14$).

The proportion of BNF-N in red clover tissue was measured in 2007 and 2008 (Fig. 5), just before the cover crop was soil incorporated in April. A management legacy effect was apparent for BNF fraction of N in red clover along the lines of the soybean response, with a downregulation of 15 to 20% associated with compost legacy, compared to conventional fertilizer managed plots (Fig. 5). We note that red clover was grown in a fertilized conventional system (CO), that from 2006 on was managed identically to that of the integrated fertilizer (IF–) system used in the soybean comparison. The difference between the two systems is that previous to 2006 the IF– system relied on banded herbicide use (one-third rate compared to CO) (Snapp et al., 2010), which in many years led to robust weed growth (Sanchez et al., 2004). In the two-factor ANOVA model test for red clover Year and Management effects, a trend was observed for a Management effect ($p = 0.07$), but not Year ($p = 0.56$), nor was the Year × Management interaction significant ($p = 0.18$). Response to management systems was similar in both years, with an average red clover fNdfa value of 56% ± 3 in the IC+ system, lower than the 68% ± 4 fNdfa observed in IF+ (HSD $p < 0.001$).

The total amount of BNF in soybean was 195 kg N ha^{-1} in 2006, a year with above-average rainfall that was well distributed throughout the growing season (Table 2). This compared to

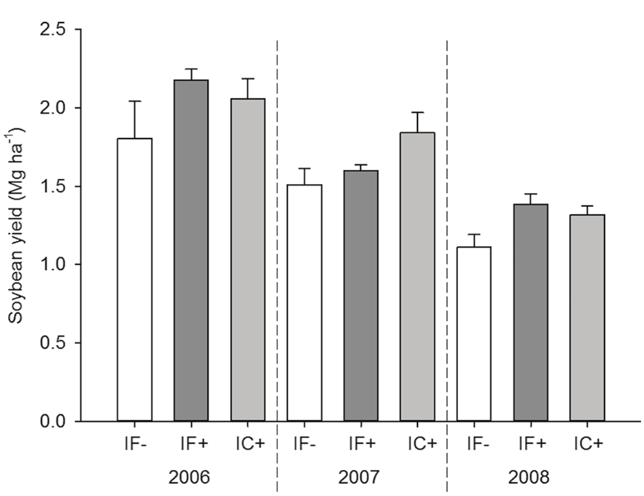

Fig. 3. Soybean (cultivar Pioneer 92M72) grain yield in three management systems (IF– = integrated fertilizer, no cover crop; IF+ = integrated fertilizer, plus cover crop; IC+ = integrated compost, plus cover crop) over the 3-yr study period in the Living Field Lab long-term experiment at Kellogg Biological Station, Michigan State University, Hickory Corners, MI. Bars represent standard errors.

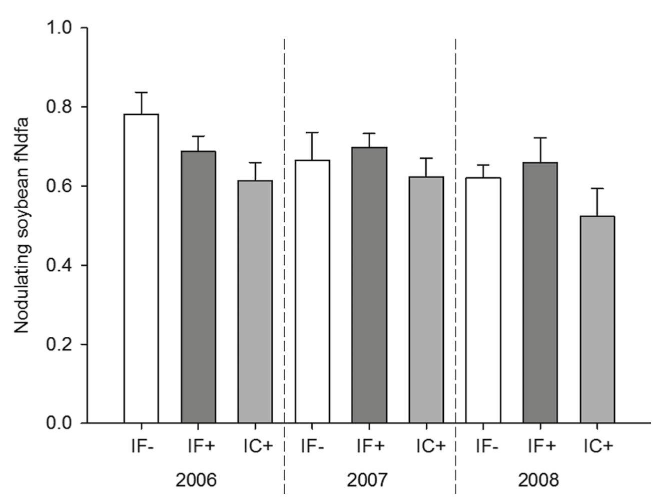

Fig. 4. Fraction of nitrogen derived from the atmosphere (fNdfa) for the nodulating soybean variety in three management regimes (IF– = integrated fertilizer, no cover crop; IF+ = integrated fertilizer, plus cover crop; IC+ = integrated compost, plus cover crop) used over the 3-yr study period in the Living Field Lab long-term experiment at Kellogg Biological Station, Michigan State University, Hickory Corners, MI. Bars represent standard errors.

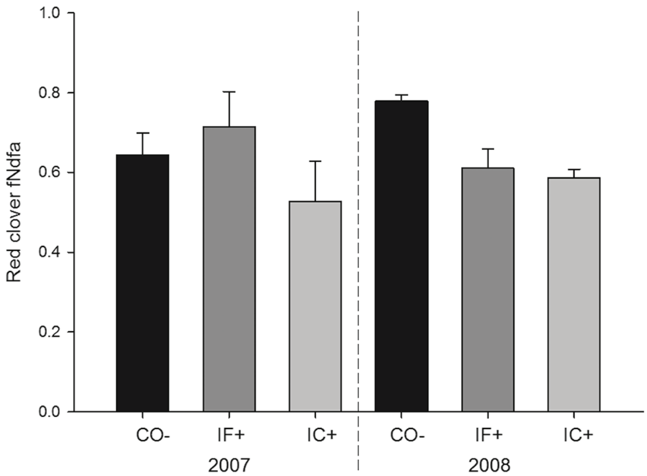

Fig. 5. Fraction of nitrogen derived from the atmosphere (fNdfa) for red clover over 2 yr in three different management regimes (CO– = conventional, no cover; IF+ = integrated fertilizer, plus cover crop; IC+ = integrated compost, plus cover crop) during the study period in the Living Field Lab long-term experiment at Kellogg Biological Station, Michigan State University, Hickory Corners, MI. Bars represent standard errors.

soybean BNF at 95 and 90 kg N ha^{-1} in 2007 and 2008, respectively. The average amount of N fixed in red clover vegetation in each year ranged from 35 to 53 kg of N ha^{-1} (Table 2), within the range of what has been observed previously in northern U.S. states although lower than some pasture-based studies (Snapp et al., 2005; Burchill et al., 2014).

DISCUSSION

There are a host of timing and placement technologies that provide incremental approaches to tightening the N cycle in fertilizer-based management; a more transformative approach to management would be to harness biological processes for internal N regulation (Drinkwater and Snapp, 2007). This long-term field study provided evidence for a legacy effect of biologically based management that enhanced soil organic matter and N supply relative to conventional management. Soil organic C and N increased in the IC+ system, which enhanced N mineralization (Fig. 2). Others have observed legacy effects of compost and cover crop management that enhances soil C and N pools, including under organic management (Bhowmik et al., 2017), and under biological management in a nearby long-term study (Grandy and Robertson, 2007). In our study, consequences of this organic matter pool build up were observed, including a reduced rate of BNF observed in plants grown in IC+ plots, in each of the 3 yr of the study. The proportion of BNF in soybean and red clover both downregulated in response to management legacy from organic nutrient sources. These findings are consistent with soil N status as an internal regulation mechanism that tightens the N cycle in agricultural system reliant on BNF, in support of sustainable N management.

Management practices performed for over a decade led to measurable gains in soil organic matter in the compost and cover crop integrated systems (Table 1). We observed a 35 to 65% increase in soil C under IC+ management, relative to IF– and CO, respectively. The labile organic matter pool was also enhanced in IC+, as indicated by POXC values (57% increase) and soil inorganic N (64% increase), relative to IF–. The soil C gains observed were similar to those observed in other long-term experiments involving crops managed with organic amendments, in the range of 12 to 50 g C m^2 yr^{-1} (Drinkwater et al., 1998; Grandy and Robertson, 2007). Relative to conventional, fertilizer-based management, enhanced pools of active soil C and mineralizable N have been previously observed in a study of Midwest organic-managed field crops (Marriott and Wander, 2006) and in a long-term experiment evaluating effects of crop diversity and organic-management (Spargo et al., 2011).

Biological Nitrogen Fixation Regulation

We report here some of the first evidence of N legacy effects in a multi-year field study. The data are consistent with downregulation of BNF in plots with a history of compost-based management, and this response occurred over multiple years with different rainfall distribution patterns. The fNdfa of soybean and red clover was lowest in the IC+ system, across multiple seasons (Fig. 4 and 5). We note that the IC+ system was also associated with high inorganic N values, mineralized from labile N and C pools (Fig. 2). The reduction in fNdfa observed is evidence for downregulation through soil N dynamics, as it was observed in management systems where soil N has accrued. The IC+ plants (relative to IF+) accumulated BNF at levels that were very similar in magnitude for both legume plants in this study. Soybean and red clover were both associated with about 54% fNdfa in plants grown in IC+ soil, compared to 68% fNdfa in plants grown in IF+ soil (Fig. 4 and 5). This is evidence for a tighter N cycle in the most ecologically integrated of the systems, as fNdfa was downregulated under

Table 2. Mean biomass, aboveground N, fraction of N derived from fixation (fNdfa) and $\delta^{15}N$ for the nodulating soybean (Nod), non-nodulating soybean (Non-nod) and clover used during this experiment from 2006 through 2008 in the Living Field Lab long-term experiment at Kellogg Biological Station, Michigan State University, Hickory Corners.

	Nodulating soybean			Non-nodulating soybean			Red clover	
Plant tissue	2006	2007	2008	2006	2007	2008	2007	2008
Aboveground biomass, Mg ha^{-1}	8.9a†	5.2b	4.9c	6.6a	3.5b	2.8c	1.9a	1.4b
Aboveground total N, kg N ha^{-1}	282a	144b	148b	150a	67.6b	61.6b	84.2a	53.6b
Aboveground $\delta^{15}N$ pods, ‰	–0.59a	–0.50ab	–0.24bc	1.81a	1.42b	1.37b	na	na
Aboveground $\delta^{15}N$ leaves and stems, ‰	–0.22a	–0.69b	–0.85c	1.32a	0.58b	0.12b	–0.67a	–0.39a
fNdfa	0.69a	0.66a	0.61b	na‡	na	na	0.63a	0.66a
N_2 from fixation, kg N ha^{-1}	195a	95.0b	90.3b	na	na	na	53.1a	35.4b
Apparent *B* value, ‰	–1.40	–1.39	–1.43	na	na	na	–1.84	–1.84

† Different letters within a plant type indicate significant differences among years using Tukey's HSD ($p < 0.05$).
‡ na, not applicable.

IC+cover crop management relative to conventional management (IF and CO).

In regards to the total amount of BNF, weather effects were marked. In the first year of the study, 2006, was a mesic year with well-distributed rainfall and outstanding growth of soybean (Table 2). The soybean BNF in 2006 was twice as much as in the other years of the study. Poorly distributed rainfall reduced plant growth and yield potential in these two later years, illustrating that BNF inputs in cropping systems can vary substantially from year to year, and are markedly influenced by growth. The importance of growth conditions on BNF inputs in agricultural systems has been noted previously by Peoples et al. (2009), yet there have been surprisingly few studies of interactions of management, soil conditions and weather, in terms of consequences for BNF in row crop systems.

Overall, soybean BNF played a major role in the N balance of this cropping system, as soybean growth over the mesic summer period of the year dominated that of red clover, which as a cover crop was confined to the poor growing conditions of fall and winter. Biological N fixation inputs from red clover in this study were in the range of 35 to 50 kg N ha^{-1}, considerably lower than that associated with soybean (although we note that all plant material was incorporated into soil from the cover crop in contrast to N grain removal from the soybean crop, which reduced N inputs by about 50%). A red clover cover crop was shown previously to contribute an apparent fertilizer N credit to corn of approximately 50 kg N ha^{-1} fertilizer, in this LFL long-term field experiment (Gentry et al., 2013). It is interesting to note that the apparent N credit to corn was similar to the amount of N derived from aboveground BNF in the red clover cover crop observed in this study.

Our findings are consistent with those reported from an on-farm study in New York, where increasing reliance on cover crops and compost was positively associated with soil inorganic N, and with downregulation of BNF (Schipanski et al., 2010). However, the results contrast with a BNF study in a Swiss long-term cropping systems FIBL trial that evaluated organic (with compost), fertilizer (with compost), and fertilizer (only) production practices. In that study, the proportion of soybean BNF measured by ^{15}N dilution was higher (53 to 47%) in the compost-amended systems vs. 32% in the N fertilizer system (Oberson et al., 2007). Management legacy effects were seen in the FIBL trial, as active C and N pools increased in systems that relied on cover crops and compost, along the lines of our study (Table 1). However, total C and N pools were not appreciatively altered by management at FIBL, and high N mineralization was observed across all management systems (possibly a legacy effect that made it difficult to ascertain management effects on BNF). In keeping with this hypothesis, overall FIBL soybean N fixation was 30 to 50%, modest rates relative to the >60% observed in our study, as well as others that have quantified soybean BNF (Oberson et al., 2007; Collino et al., 2015). Clearly, the interactions of weather and management practices on BNF are complex, and more studies are needed that tackle this complexity over time and space (Table 2).

In an on-farm study Schipanski et al. (2010) found a remarkably wide range of BNF fixed by soybean: from 40 to 220 kg N ha^{-1} (^{15}N natural abundance method), and a high correlation of BNF with biomass. This highlights the role of plant growth in determining the total amount of BNF in agricultural systems (in concurrence with our findings, Fig. 4). Soybean reliance on N_2 fixation ranged from 36 to 82%, and a positive relationship was found between N uptake by soybean and proportion of N from BNF (Schipanski et al., 2010). We note, however, in this on-farm study other soil properties, for example, texture and total N, were stronger BNF predictors than soil inorganic N (Schipanski et al., 2010). High variability of BNF associated with soil environmental properties has been observed previously (Herridge and Brockwell, 1988; Schipanski et al., 2010; Collino et al., 2015), but our study highlights that seasonal effects can be even more important than soil properties, with twofold greater total amount of BNF fixed under mesic weather conditions compared to years with unfavorable weather.

Soybean Yield

Gains in soybean yields relative to conventional fertilizer-only management (IF–) were observed for the biologically integrated systems that relied on judicious fertilizer use combined with cover crops (IF+) or with compost (IC+), as shown in Fig. 3. This is similar to findings of higher yields under organic, compost based management in a European long-term experiment (Oberson et al., 2007), although a 20% reduction in soybean yield was observed under organic management in the mid-Atlantic region of the United States, compared to conventional (Cavigelli et al., 2008). Year effects on yield were high as well in our study, following the same pattern as that observed for total amount of BNF: 2008 > 2007 > 2006. In the "Scale up" experiment that involves 27 fields at the Kellogg Biological Station (where the LFL trial is located) a strong seasonal weather effect was also observed, where soybean yields varied from ~1 to 3 MG ha^{-1} depending on the year (Kravchenko et al., 2017).

CONCLUSIONS

Our findings provide some of the first evidence of internal regulation mechanisms operating robustly for a field crop sequence. We observed a consistent response over three seasons and two plant species. Down-regulation of BNF occurred in both soybean and red clover plants grown in soils with enhanced labile organic matter, whereas soybean yield was maintained or enhanced. The total amount of N fixed in both conventional and alternative field crop production systems was largely a product of plant growth, driven by seasonal precipitation patterns. The enhanced total amount of atmospheric-derived N in a mesic rainfall year should not be overlooked and this has implications for the N balance across agricultural landscapes. Future research is required to understand how feedback mechanisms controlling BNF interact with weather, management and environmental site properties, as these may be of substantial magnitude and influence agricultural sector impacts on global reactive nitrogen.

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