

## Use of N immobilization to tighten the N cycle in conventional agroecosystems

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**Abstract.** Soils in conventional agroecosystems are purposely held in a nitrogen (N)-saturated state to maximize crop yields. Planting winter annual cover crops when fields are usually fallow has been proposed to ameliorate N losses from soils. In this study we introduced winter annual cover crops into an N rate study with plots fertilized at 0, 34, 67, 101, 134, 168, and 202 kg N/ha in maize (*Zea mays* L.) to determine how winter annual cover crops affect yields, N<sub>2</sub>O and NO<sub>3</sub><sup>-</sup> fluxes, and N pools. At the six-leaf stage and during flowering, incorporation of cover crop into soil resulted in a 30% reduction in maize biomass. Three weeks after fertilization, KCl-extractable soil mineral N was 75–87% lower in cover-cropped soils than in no-cover soils, indicating that N had been immobilized in the cover-cropped soils. At physiological maturity, there was no difference between cover and no-cover treatments in crop yield, which was maximized at 9 Mg/ha in 2006 and 7 Mg/ha in 2007. Where N rates exceed crop requirements, cover crop incorporation may reduce N exports as NO<sub>3</sub><sup>-</sup> and N<sub>2</sub>O. Tighter N cycling in conventional agroecosystems could be fostered by matching N rates to the amount of N removed with grain and using N immobilization to retain N and support yields. If N immobilization is viewed as a means for efficient fertilizer N use rather than a process that decreases crop productivity, growers might be more willing to adopt cover-cropping practices.

*Key words:* cereal rye; cover crop; maize; nitrate; nitrogen rate study; nitrous oxide; winter wheat.

### INTRODUCTION

Nitrogen (N) saturation theory predicts that when N inputs to an ecosystem are greater than plant and microbial demand, exports of multiple forms of N, many of which are pollutants, will be stimulated. Farming systems are often intentionally held in an N-saturated state to maximize productivity. Many studies have demonstrated the potential for leaching losses of nitrate (NO<sub>3</sub><sup>-</sup>) when farm fields are fertilized at N levels that are greater than those required to support yields, often with nonlinear responses (Bergström and Brink 1986, Steinhilber and Meisinger 1995, Andraski et al. 2000, Power et al. 2000, Shepherd and Chambers 2007). In addition, N availability is considered a major controller of nitrous oxide (N<sub>2</sub>O) emissions in agricultural soils (Mosier et al. 2006, Stehfest and Bouwman 2006), increasing abruptly at N levels that are above those required for maximum maize yields (McSwiney and Robertson 2005, Mahli and Lemke 2007).

For an ecosystem with N-saturated soils, strategies that increase plant and microbial N uptake should aid in decreasing N exports of all types, and recycling of the N

held in plant and microbial pools can reduce fertilizer requirements for subsequent crops (Drinkwater and Snapp 2007). Nitrogen accumulation in winter annual cover crops planted after crop harvest has been proposed as a means to reduce N losses. Nonleguminous cover crops established in the fall will begin to remove any residual fertilizer N that was not utilized during the growing season (Rasse et al. 2000, Weinert et al. 2002, Strock et al. 2004, Vos and van der Putten 2004). This is important when there is substantial rainfall after the crop has senesced, particularly for sandy soils, as fertilizer N remaining in soil is then subject to leaching loss (MacDonald et al. 2005). The majority of cover crop growth and N uptake will occur in the spring and transpiration may provide additional protection against leaching losses (Munawar et al. 1990, Corak et al. 1991, Raimbault and Vyn 1991). When covers are chemically or mechanically killed and incorporated early enough in the spring, N in cover crop residues will be rereleased through decomposition and mineralization to the new crop being established (Jackson 2000, Crandall et al. 2005).

In this study we examined how introduction of a winter annual cover crop into a maize agroecosystem affects N pools and fluxes under N-deficient, N-sufficient, and N-saturated conditions on sandy loam soils at the W. K. Kellogg Biological Station in

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southwest Michigan, USA. We hypothesized that plant/microbe-available N pools would be enhanced by incorporation of a cover crop through N recycling, that N immobilization would be minimized by timely killing of the cover crop before planting, and that the magnitude of  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$  losses would be altered when a winter cover crop is introduced into the maize ecosystem. Our objectives were to determine how growing and incorporated dead cover crop affect N pools and fluxes across a gradient of N availability, specifically soil N pools over the growing season,  $\text{NO}_3^-$  leaching,  $\text{N}_2\text{O}$  fluxes, and plant N uptake into winter annual cover crop and maize plants.

## METHODS

### Site description

We conducted this study at the W. K. Kellogg Biological Station (KBS) located in southwest Michigan, USA, 50 km east of Lake Michigan ( $42^\circ 24' \text{ N}$ ,  $85^\circ 24' \text{ W}$ , elevation 288 m) on soils developed from glacial outwash deposited 12 000 years ago. Soils are mainly of the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs), Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) and Miami (fine-loamy, mixed mesic Typic Hapludalfs) series, which all occur on our site (J. R. Crum and H. P. Collins, *available online*).<sup>5</sup> The area receives  $\sim 90$  cm of precipitation annually, about half as snow, and the mean annual temperature is  $9.7^\circ\text{C}$ . Further site and soils descriptions for KBS are *available online* (see footnote 5).

### Experimental design

In 2006, we established this study as a split-split block design with four replicates in which a maize–maize–soybean rotation was subjected to two different winter management systems, cover crop vs. no cover crop, and N fertilization levels randomly assigned to  $9 \times 9$  m subplots at 0, 34, 67, 101, 134, 168, and 202 kg  $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  (Fig. 1). Maize was planted in 2006 and 2007 in rows at 0.76-m intervals, resulting in 12 rows per block, six rows each in cover and no cover. A field previously managed as a maize–soybean–wheat rotation had been planted to winter wheat (*Triticum aestivum* L.; Pioneer 25R37) at 168 kg seed/ha on 5 October 2005. While in the maize–soybean–wheat rotation, maize was fertilized at 140 kg  $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ , wheat at 45 kg  $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ , and soybean was not fertilized. Wheat planted in 2005 was used as a cover crop in the first year of the study, and cereal rye (*Secale cereale* L.) was used subsequently as the cover crop. No-cover plots were established in early spring (1 April 2006) by chemically killing (glyphosate at 0.46 kg active ingredient/ha) wheat and weeds, which were of negligible biomass, on half of each block. On 9 May 2006, the wheat plants that had

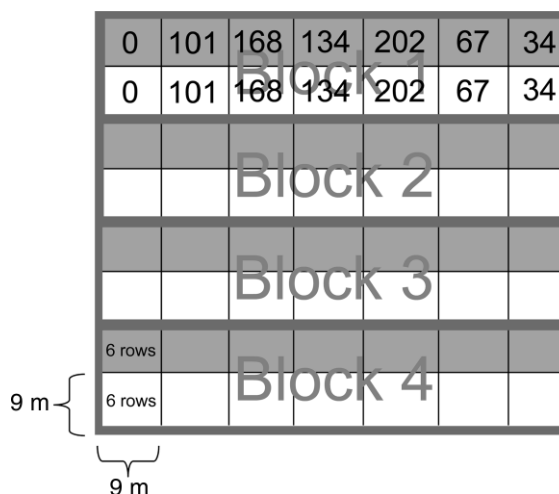


FIG. 1. Experimental design, where gray represents the cover crop on half of each block and white represents no-cover treatment. Numbers in each subplot for Block 1 represent the randomly assigned nitrogen fertilizer treatments of 0, 34, 67, 101, 134, 168, and 202 kg N/ha. To simplify the figure, fertilizer treatments are not shown for other blocks.

been allowed to grow as the cover crop on the cover plots were killed (glyphosate at 0.46 kg active ingredient/ha) and turned into the soil 15 days later during site preparation for planting. Cereal rye was planted on 27 November 2006 at 134 kg seed/ha. In 2007, rye cover and weeds in the cover crop plots were killed on 8 May and incorporated six days later.

### Agronomy

Maize (Pioneer 36W66) was planted without starter N fertilizer at 81 543 seeds/ha (25 May 2006, 21 May 2007) and thinned to 69 160 plants/ha at the six-leaf stage of development (26 June 2006, 21 June 2007). On 28 June 2006 and 22 June 2007, fertilizer N ( $\text{NH}_4\text{NO}_3$ ) was applied by hand and then incorporated into the subplots at seven rates from 0 to 202 kg N/ha. Nitrogen rates were randomly assigned within each of the four blocks for a total of 56 subplots (Fig. 1). Nitrogen fertilizer treatments were assigned to the same subplots in 2006 and 2007. S-metolachlor, mesotrione, and atrazine were applied to control weeds on 26 May 2006 and 21 May 2007. Table 1 summarizes the timing of management practices for 2006 and 2007. Other agronomic management followed best management practice for conventional farm fields in the local area (information *available online*).<sup>6</sup>

### Plant measurements

Cover crop biomass samples were taken before the plants were killed to determine cover crop N uptake over the fall and into spring. In both years, aboveground biomass was harvested from a  $0.5 \times 0.5$  m quadrat, dried

<sup>5</sup> ([http://lter.kbs.msu.edu/about/site\\_description/soils.php](http://lter.kbs.msu.edu/about/site_description/soils.php))

<sup>6</sup> (<http://lter.kbs.msu.edu/protocols/104>)

TABLE 1. Timing of farming practices for 2006 and 2007 growing seasons.

Operation	2006	2007
Cover planted	5 October 2005†	27 November 2006‡
Cover killed	9 May 2006	8 May 2007
Cover incorporated	24 May 2006	14 May 2007
P and K fertilizer applied§	6 May 2006	3 May 2007
Maize planted#	25 May 2006	21 May 2007
N fertilizer applied††	28 June 2006	22 June 2007
Maize harvested	15 September 2006	25 and 26 September 2007

† Winter wheat was planted as cover at a rate of 168 kg seed/ha.

‡ Cereal rye was planted at a rate of 134 kg seed/ha.

§ Fertilizer applied at the following rates: P, 50.4 kg/ha; K, 78.4 kg/ha.

# Maize planted at 81 543 seeds/ha using varietal Pioneer 36W66.

†† Fertilizer was applied as  $\text{NH}_4\text{NO}_3$ .

at 60°C to constant mass, and the final mass was recorded. Wheat cover crop aboveground biomass was assessed (8 May 2006) just before it was killed by sampling the cover plots at three random locations in each block for a total of 12 cover-plant samples. On 8 May 2006, the no-cover plots were still bare due to the herbicide application in April and were not sampled.

On 10 and 11 May 2007, two aboveground biomass samples were taken per subplot for a total of 112 samples. In 2007, aboveground biomass in the cover plots was comprised of rye cover crop and weeds, and there were weeds present in the no-cover plots. After cover crop and weeds were sampled in 2007, a composite of three root soil cores (three 5 cm diameter by 20 cm deep cores) was taken from each quadrat in 0, 101, and 202 kg N/ha subplots in both cover and no-cover for a total of 48 samples. Root soil cores were placed on a 4-mm mesh screen and flushed with water to remove the soil. Roots were picked off the screen and then dried at 60°C for 48 h.

Maize plant samples were taken throughout the field season to assess crop N uptake. Rows three and four in each plot were designated as yield rows, where all maize plant samples were taken at physiological maturity (Fig. 1). Growing season maize plant samples were taken from rows two and five to minimize changes in plant density. A composite sample of eight maize plants was collected from rows two and five on the cover and no-cover sides of each block at 12 random locations for a total of 24 maize samples at the six-leaf stage of development (26 June 2006) before the fertilizer treatments were imposed. After fertilization, a composite sample of four maize plants was collected from rows two and five in each of the 0, 67, 101, 134, and 202 kg N/ha subplots on both the cover and no-cover sides on 3 August 2006 for a total of 40 samples, dried at 60°C to constant mass, and the mass was recorded.

At physiological maturity, the aboveground portions of four maize plants from rows three and four of each plot were sampled to determine crop N uptake (Gentry et al. 1998). The biomass samples were divided into three fractions: (1) husk, cob, shank, and tassel (reproductive support); (2) leaf and stem; and (3) grain. The leaf and

stem fraction was weighed fresh, shredded, and a subsample was taken for the remainder of the analyses. After whole-plant sampling, the remaining ears from rows three and four were collected by hand, shelled, and the grain was weighed. Cobs from the whole-plant samples were added to the reproductive support fraction. The grain mass from the whole-plant samples was added to the mass obtained by sampling the remaining ears to determine grain yield. All three fractions of the four-plant samples were dried at 60°C to constant mass, and then weighed to determine biomass. For this study, we report the total of all three fractions in aboveground biomass and in aboveground biomass N.

Aboveground wheat cover crop, six-leaf stage maize (26 June 2006), post-pollination maize (3 August 2006), and plants collected at physiological maturity were ground to pass a 1-mm screen in a Christy-Turner 8-inch (20.3 cm) Lab Mill (Ipswich, Suffolk, UK) and then analyzed for total C and N using a Carlo-Erba NA 1500 CNS (Carlo-Erba, Milan, Italy).

Maize chlorophyll meter readings were taken twice during the growing season 10–14 days after pollination (31 July 2006) and 24–28 days after pollination (16 August 2006) to determine N status for the growing maize plants. We used a Minolta SPAD 502 meter (Konica Minolta, Ramsey, New Jersey, USA) to take measurements on the leaf above the ear in a spot that was unblemished and without holes. Thirty leaves were measured for each plot and then averaged.

#### *Nitrogen availability*

Nitrogen availability was assessed three ways: (1) N mineralization potential of soils collected in early June; (2) ion-exchange resin strips, an index that integrates effects of soil N levels and soil water content on N availability for the period deployed; and (3) N in aboveground maize biomass at physiological maturity for plants grown without N fertilizer. Methods 1 and 3 provide estimates of soil N without the impact of fertilizer N, while method 2 includes the impact of fertilizer N.

N mineralization potential was determined for soils collected on 12 June 2006 and 30 May 2007, which occurred after the cover/no-cover treatments had been imposed on the field and the maize had been planted, but before N fertilizer had been applied. In 2006, we sampled the cover and no-cover treatments at three random locations in each block for a total of 12 cover and 12 no-cover soil samples, and in 2007 all 56 subplots were sampled. At each location, we took a composite sample of five 0–25 cm cores and stored them at 4°C until they were processed within 24 h. Soils were sieved to 4 mm and homogenized before two 10-g subsamples and a sample for soil moisture were weighed. For the initial sample, 100 mL of 1 mol/L KCl were added immediately to one of the two subsamples, the cups were shaken for 1 min, and then allowed to settle overnight. The next day, the cups were shaken again, allowed to settle for 1 h, filtered through Whatman number 1 filter paper into scintillation vials, and then frozen until analysis. Soil samples weighed for gravimetric water content were placed into a drying oven held at 60°C, dried for 48 h, and weighed again to determine water content and dry weight for the soil N analyses. For the final sample, the second 10-g subsample was corrected to 19% soil moisture (field capacity for KBS soils), placed in an incubator held at 25°C for 28 d, and then extracted in 1 mol/L KCl as described for the initial sample. Nitrate and  $\text{NH}_4^+$  were determined in the soil extracts using the SmartChem 140 discrete analyzer (Westco Scientific, Danbury, Connecticut, USA) for  $\text{NO}_3^-$  using hydrazine reduction followed by development of an azo dye and  $\text{NH}_4^+$  using the phenolate method. Nitrogen mineralization potential was calculated as the difference between total mineral N ( $\text{NO}_3^- + \text{NH}_4^+$ /g dry soil) from the incubated soil and the total mineral N from the soil that was extracted immediately. We present the data on a 28-day basis.

On 17 July 2006, we sampled soils from all 56 subplots to determine soil N availability shortly after fertilization. Soil sampling, processing, and analysis were done as for the initial samples described in the previous paragraph. The mass of soil N per hectare was calculated based on the bulk density ( $\text{BD} = 1.62$ ) for the 0–25 cm depth interval of soils from the KBS Long-Term Ecosystem Research (LTER) conventional treatment, which is adjacent to our site and follows similar tillage practices.

We monitored N availability from 18 June to 11 October 2006 and 14 May through 17 October 2007 using ion-exchange resin strips in the 0, 67, 101, 134, and 202 kg N/ha subplots, in cover and no-cover treatments, for a total of 40 determinations at each sampling. Cation- and anion-exchange membranes (Ionics, Waterville, Massachusetts, USA) were cut into 2.5 cm by 10 cm strips, charged (shaken 1 h in 0.5 mol/L HCl and 5 h in 0.5 mol/L  $\text{NaHCO}_3$ ), and rinsed with deionized water. Anion and cation strips were inserted into the soil by making vertical slots in the soil, placing the strips into the soil, and firmly closing the slots so that

the soil and the strips were in contact. After two weeks, the paired anion and cation strips were removed from the soil, rinsed with deionized water to remove any adhering soil, placed together in a vial for return to the laboratory, and new strips were placed in an adjacent location. In the laboratory, 70 mL of 2 mol/L KCl was added to each vial containing the cation and anion strip pair, shaken for 1 h, decanted into a scintillation vial, and frozen until analysis. Before reuse, the strips were recharged as described with HCl and  $\text{NaHCO}_3$ . Ion-exchange strip extracts were analyzed using a SmartChem 140 discrete analyzer for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$ , as described for the soil extracts for  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , and removing the  $\text{NO}_3^-$ -reduction step for  $\text{NO}_2^-$  analysis.

#### *Nitrogen export: nitrous oxide and soil water nitrate sampling and analyses*

For N export measurements, we studied levels of N addition that represent N deficient (0 kg N/ha), sufficient (101 kg N/ha), and excessive (202 kg N/ha) conditions for this site. Our N-sufficiency designations are based on yield responses from previous N rate studies at the KBS-LTER site wherein maize grain yields increased with N rate up to 101 kg N/ha, above which there were no further gains in yield (McSwiney and Robertson 2005).

In order to describe potential effects of cover crop incorporation into soil and cover crop growth on N export, we measured  $\text{N}_2\text{O}$  fluxes in 2006 and 2007. In 2006, we focused on  $\text{N}_2\text{O}$  fluxes after cover crop incorporation into soil by making measurements two times before fertilization of the maize crop, and then approximately once every two weeks until fluxes diminished in October. Sampling dates with days after fertilization and total rainfall for seven days previous to measurement were: 20 June (–8 days, 10.8 mm), 27 June (–1 day, 17.1 mm), 12 July (14 days, 10.5 mm), 25 July (28 days, 29.5 mm), 8 August (42 days, 35.3 mm), 24 August (59 days, 40.6 mm), and 8 September (75 days, 0 mm). For 2007,  $\text{N}_2\text{O}$  flux measurements were targeted to assess the effects of cover crop growth, cover crop incorporation into soil, and fertilization by sampling all 56 plots: (1) when a rye cover crop was growing on 10 May (prefertilization, 5.7 mm), (2) after the rye had been killed and incorporated and the maize crop had been planted on 29 May (–22 days, 29.9 mm), and (3) after N fertilizer had been applied on 20 July (30 days, 17.1 mm). Nitrous oxide measurements and analyses were conducted using a static-chamber method used for earlier studies at the KBS-LTER site (e.g., Robertson et al. 2000). We installed one chamber base made from polyvinyl chloride (PVC) pipe in each plot (25 cm diameter  $\times$  10 cm height). They remained in the field between agronomic operations, with lids attached only for measurement periods of up to two hours. Four gas samples were taken through a sampling port over the incubation period by flushing and then pressurizing 11-

mL autosampler vials in 2006 and 6-mL vials in 2007. Gas vials were then taken to the laboratory for analysis within 36 h. Nitrous oxide measurements were made in all four blocks at each of the seven N levels, with and without cover crops on each sampling date.

An autosampler (Gilson Sample Changer 221-222, Villiers-Le-Bel, France) was used to introduce chamber headspace samples into a gas chromatograph (Hewlett Packard 5890 Series II, Rolling Meadows, Illinois, USA), where N<sub>2</sub>O was separated on a Porapak QS column (1.8 m, 80/100 mesh, held at 80°C) and quantified with a <sup>63</sup>Ni electron capture detector at 350°C. Carrier gas was argon/methane (90/10).

Nitrate movement past the rooting zone was monitored using gravity lysimeters from 29 September 2006 through 24 October 2007. We installed lysimeters in cover and no-cover treatments that were fertilized at 0, 101, and 202 kg N/ha for a total of 24 samplers. The lysimeters consisted of 5 cm diameter PVC pipe with a 42 cm length of screen with 0.6-cm (1/4-inch) slots on the upper half of the pipe's circumference. Screen was located on the PVC pipe such that when it was installed into the soil at a 45° angle, the screen would capture soil water that had moved to a depth of 110 cm, which corresponds with the C horizon in our field sites. Window screen and sediment trapping material were placed inside the slotted section of the PVC pipe to trap any large particles of soil that passed through the slots in the pipe. Water coming into the pipe was trapped at the bottom in a reservoir, which we sampled by running 0.32-cm (1/8-inch) nylon tubing to the bottom of the reservoir and routing the tubing out into the alleyways between fields. We sampled soil water on an event basis by applying a vacuum to 1-L jars to draw the water out of the reservoir. All samples were weighed upon return to the laboratory and filtered. Samples and blanks were filtered through Whatman number 1 filter paper into scintillation vials and frozen until analysis. Soil solutions were analyzed for NO<sub>3</sub><sup>-</sup> as described under *Nitrogen availability*.

#### *Data analysis*

For plant and soil samples that were collected before N fertilization, we tested for the effect of the cover crop using a one-way ANOVA (SYSTAT 7.0; SPSS 1997). For all response variables collected after fertilization we used PROC MIXED in SAS 9.1 (SAS Institute 2002) to determine whether the effects of cover crop, N fertilizer level, and their interaction were significant. We log-transformed N<sub>2</sub>O flux, aboveground biomass N at harvest, and soil NH<sub>4</sub><sup>+</sup> before statistical analysis to meet assumptions about normality. Over the period that we monitored NO<sub>3</sub><sup>-</sup> leaching, we rarely had samples in all replicates for each treatment and often just one, most likely due to spatial variability in hydrologic conductivity. We present the NO<sub>3</sub><sup>-</sup>-N masses collected for each individual lysimeter and limit ourselves to a qualitative discussion of the data for the period monitored.

## RESULTS

### *Weather, cover crop, and maize biomass*

Rainfall was distributed evenly during the growing season of 2006, while there was a month and a half long drought in 2007 (Fig. 2). Total rainfall from 1 April to 30 September was 575 mm in 2006 and 429 mm in 2007. The 30-year average for growing season rainfall for our field site is 532 mm.

Incorporation of the wheat cover crop into the soil in 2006 provided 1.28 Mg/ha of aboveground biomass and 23.5 kg N/ha at a C:N ratio of 27, while the no-cover plots remained bare (Table 2). In 2007, rye and weeds in the cover plots provided a total of 0.78 Mg/ha of aboveground biomass, and weeds in the no-cover plots supplied 0.88 Mg/ha of aboveground biomass when incorporated into the soil (Table 2). Contributions of N from incorporated biomass were 14.4 kg/ha total for rye and weeds in cover plots and 13.9 kg/ha for weeds in the no-cover plots (Table 2). Belowground biomass was 0.46 Mg/ha in the cover plots and 0.20 Mg/ha in the no-cover plots in 2007 (Table 2).

During much of the growing season in 2006 we saw reductions in aboveground maize biomass in cover crop plots relative to no-cover plots. Maize aboveground biomass collected on 26 June 2006 was 0.24 Mg/ha in no-cover plots, which was greater than the 0.17 Mg/ha found in cover-cropped plots ( $P = 0.001$ ; Table 2). On the same date, N accumulation in aboveground maize biomass was 9 kg N/ha in no-cover plots and 5.5 kg N/ha ( $P = 0.00003$ ; Table 2) in cover-cropped plots. On 2 August 2006, maize aboveground biomass differed significantly due to the different rates of N applied ( $P = 0.006$ ), and maize aboveground biomass was still significantly less on the cover-cropped plots than on no-cover plots ( $P = 0.018$ ), being most pronounced where no N fertilizer was applied (Fig. 3a). Likewise, total N accumulated in aboveground maize biomass on 2 August was significantly greater in no-cover plots than in cover plots ( $P = 0.046$ ) and exhibited a significant response to added N ( $P < 0.0001$ ; Fig. 3b).

Measurements of maize N status at critical points during the growing season reflected the patterns seen in biomass accumulation. Increasing fertilizer addition resulted in significant increases in chlorophyll meter readings ( $P < 0.0001$  for 31 July 2006 and  $P < 0.0001$  for 16 August 2006). Plants on cover-cropped plots were more N deficient compared to no-cover plots on 31 July in 0 kg N/ha subplots ( $P = 0.0171$ ) and on 16 August in 0 kg N/ha ( $P = 0.0404$ ) and 34 kg N/ha subplots ( $P = 0.0002$ ) (Fig. 4). At N rates of 67 kg N/ha or greater, there was no difference in maize N status between cover and no-cover plots (Fig. 4).

At physiological maturity, there was no significant difference in total aboveground biomass between maize grown on cover-cropped soils and those without cover crops ( $P = 0.2101$  for 2006 and  $P = 0.9815$  for 2007; Fig. 5c, d) in 2006 or 2007. There was a significant response

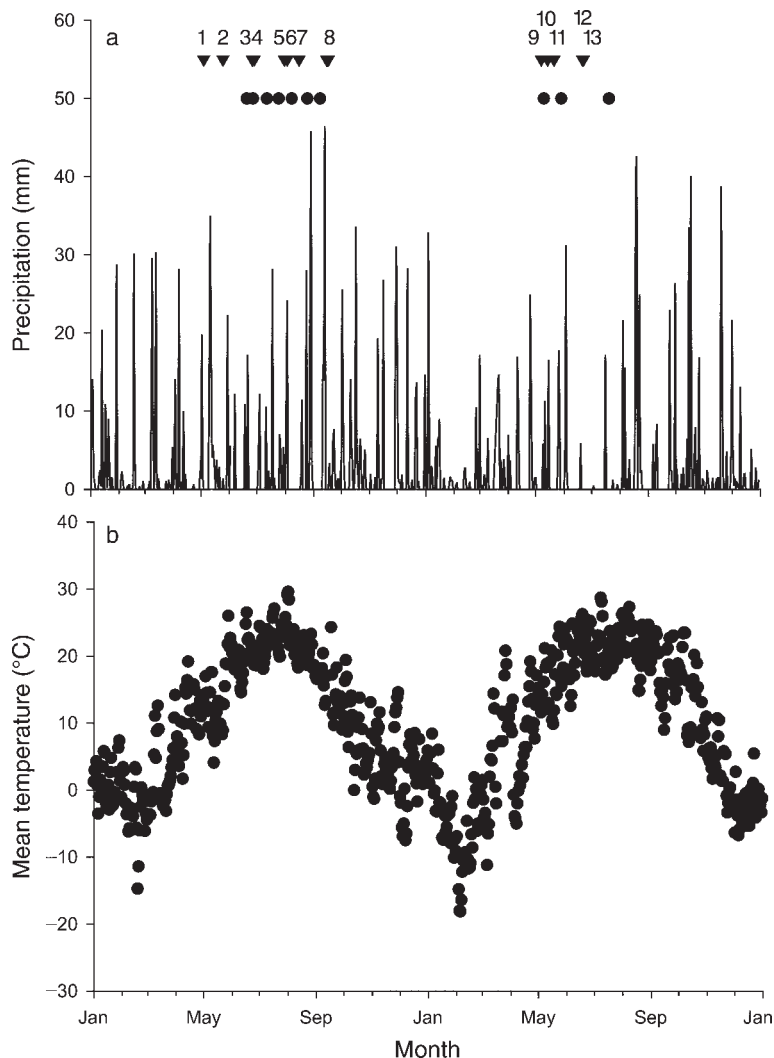


FIG. 2. (a) Precipitation and field operations/sampling events and (b) temperature for 2006 and 2007. In panel (a), triangles represent the dates when major farming practices were conducted and when plant biomass was sampled, circles represent N<sub>2</sub>O-flux sampling, and the vertical lines represent the total rainfall for that day. Events were: (1) cover crop sampled, (2) cover crop incorporated and maize planted, (3) six-leaf stage maize sampled, (4) fertilizer applied and incorporated, (5) maize chlorophyll measurement, (6) maize plants sampled, (7) maize chlorophyll measurement, (8) maize plant harvest at physiological maturity, (9) cover crop sampled, (10) cover incorporated, (11) maize planted, (12) six-leaf stage maize sampled, and (13) fertilizer applied and incorporated.

TABLE 2. Biomass and N content of cover crop and early growing season maize (values in parentheses are standard errors).

Type and date	Biomass type	Biomass (Mg/ha)		Total N (kg N/ha)	
		Cover plot	No-cover plot	Cover plot	No-cover plot
Cover crop and weeds					
20 May 2006	wheat	1.281 (0.088)		23.41 (0.6)	
7 May 2007	rye	0.282 (0.016)		6.643 (0.381)	
	weeds	0.494 (0.03)	0.88 (0.026)	7.744 (0.479)	13.94 (0.365)
	roots	0.462 (0.054)	0.197 (0.046)		
Six-leaf stage crop					
26 June 2006	maize	0.172 (0.01)	0.245 (0.017)	5.5 (0.33)	9 (0.59)
20 June 2007	maize	0.131 (0.003)	0.153 (0.004)	3.372 (0.097)	3.896 (0.102)

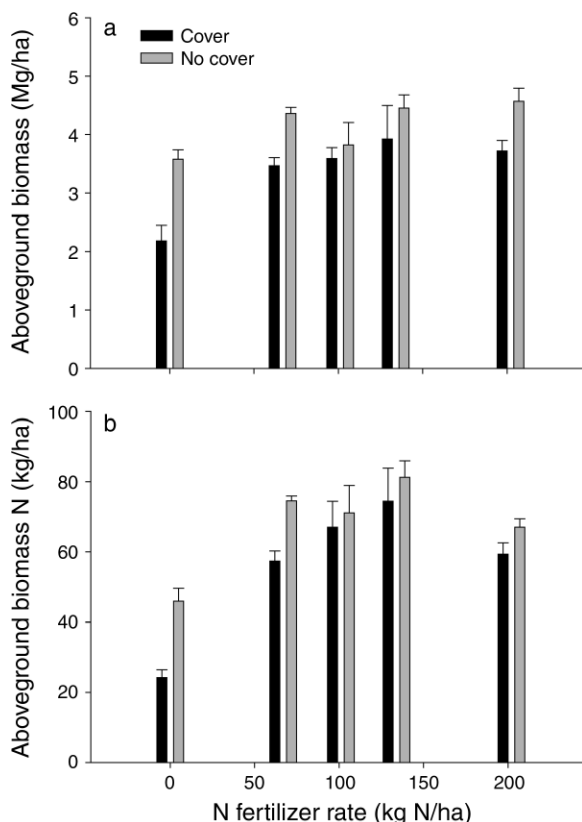


FIG. 3. (a) Maize biomass (mean + SE) and (b) total N in aboveground biomass for maize plants collected on 2 August 2006 at each of five N application rates, with and without cover crop incorporated into the soil.

to the amount of N fertilizer added ( $P < 0.0001$  for 2006 and  $P < 0.0003$  for 2007; Fig. 5c, d) and no interaction between the effects of cover and N rate ( $P = 0.0593$ ). Patterns in total N accumulated in aboveground biomass were similar to those for total aboveground biomass. There was no difference in aboveground N accumulation between cover and no-cover plots ( $P = 0.1573$ ; Fig. 5e, f) and significant differences in the accumulation of N in response to different N rates ( $P < 0.0001$ ; Fig. 5b). Grain yield responses to fertilizer additions were similar to those of total aboveground biomass (Fig. 5a, b).

#### N availability

Nitrogen mineralization potential of soils collected on 12 June 2006 indicated a greater potential for N mineralization in soils that were amended with cover crops ( $24 \text{ kg N} \cdot \text{ha}^{-1} \cdot [28 \text{ d}]^{-1}$ ) than in no-cover soils ( $15 \text{ kg N} \cdot \text{ha}^{-1} \cdot [28 \text{ d}]^{-1}$ ) ( $P = 0.012$ ). However, the initial concentrations of  $\text{NO}_3^-$  in the no-cover soils were about twice those in the cover soils ( $11.2 \text{ g/kg}$  vs.  $5.6 \text{ g/kg}$ ;  $P = 0.000001$ ), while the  $\text{NH}_4^+$  concentrations were similar ( $1.0 \text{ g/kg}$  vs.  $1.1 \text{ g/kg}$ ;  $P = 0.1715$ ). In 2007, net mineralization potential for soils collected on 30 May was not significantly different between cover and no-cover soils with a release of  $14 (\pm 1.9) \text{ kg N/ha}$  for cover soils and of  $8 (\pm 1.9) \text{ kg N/ha}$  for no-cover soils ( $P = 0.06$ ). Initial soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  for the soils collected on 30 May 2007 were similar, with cover  $\text{NO}_3^-$  at  $6.7 (\pm 0.2) \text{ g/kg}$ , no-cover  $\text{NO}_3^-$  at  $6.9 (\pm 0.2) \text{ g/kg}$ , cover  $\text{NH}_4^+$  at  $2.4 (\pm 0.1) \text{ g/kg}$ , and no-cover  $\text{NH}_4^+$  at  $2.1 (\pm 0.1) \text{ g/kg}$ .

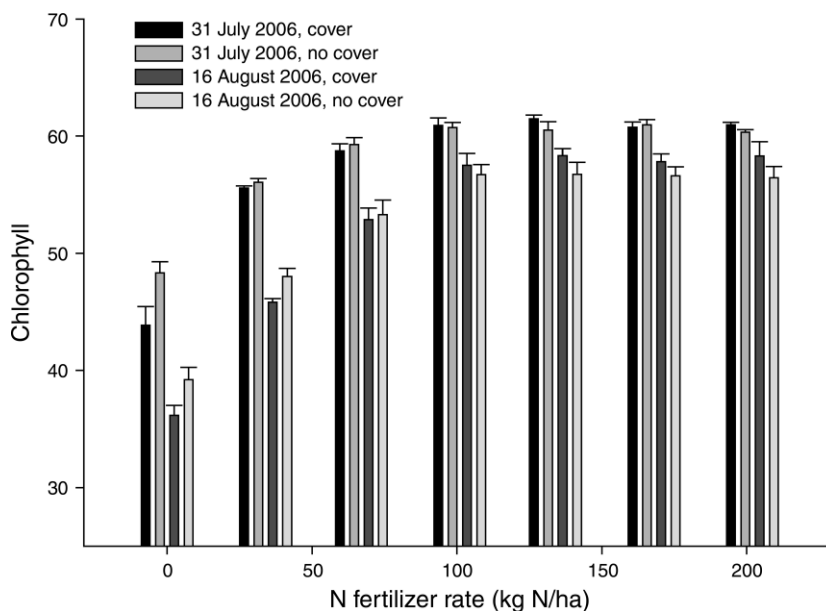


FIG. 4. Maize chlorophyll meter readings (mean + SE) taken 31 July (first pair of bars at each fertilizer rate) and 18 August 2006 (second pair of bars at each fertilizer rate) with cover and without cover crops incorporated into the soil at each of the seven N rates.

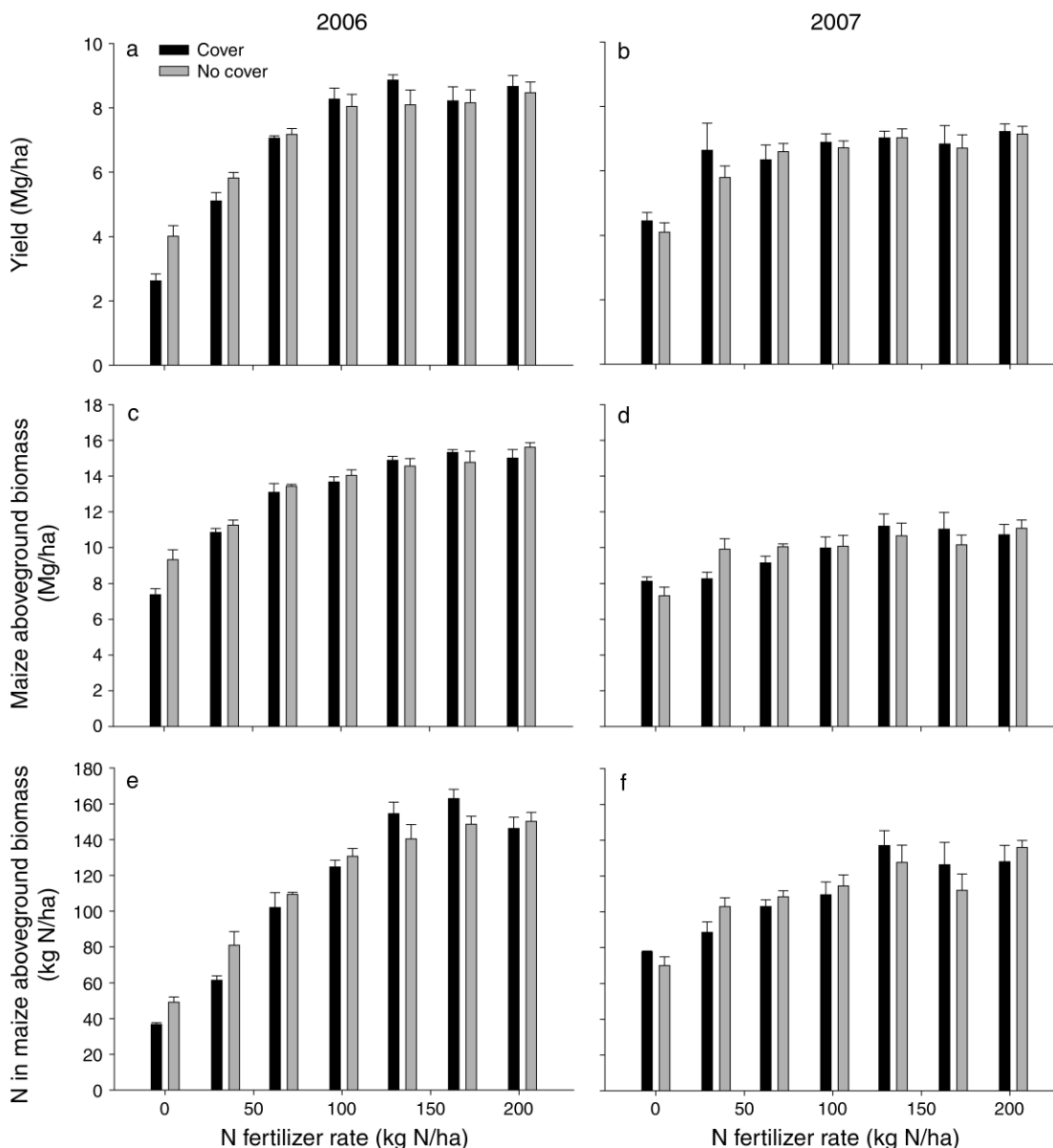


FIG. 5. (a, b) Maize grain yields in 2006 and 2007, (c, d) aboveground maize biomass at harvest in 2006 and 2007, and (e, f) N accumulated in aboveground maize biomass at harvest in 2006 and 2007. Values are means + SE.

Three weeks after N fertilization (17 July 2006), no-cover soils had much higher concentrations of inorganic N compared to cover soils, for all N rates except the unfertilized plots ( $P = 0.0793$  for  $\text{NO}_3^-$  and  $P = 0.0027$  for  $\text{NH}_4^+$ ; Fig. 6a). Ammonium dominated in the no-cover soils. Increasing N additions still affected soil N levels at this point in the growing season, although the effect was subtle for cover crop soils ( $P = 0.0612$  for  $\text{NO}_3^-$  and  $P < 0.0001$  for  $\text{NH}_4^+$ ; Fig. 6a). The relationship between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  further illustrates the dominance of  $\text{NH}_4^+$  at this point in the growing season and overall lower mineral N concentrations in the cover soils relative to the no-cover soils (Fig. 6 inset).

In no-cover soils, there was little increase in  $\text{NO}_3^-$  concentrations at higher N rates despite large increases in  $\text{NH}_4^+$  (Fig. 6 inset). Ion-exchange resin strips that were deployed for the two weeks before the 17 July soil sampling also reflect the dominance of  $\text{NH}_4^+$  at this time of the growing season (Fig. 6b).

Cumulative N availability, determined using ion-exchange resin strips deployed for two-week periods, did not differ between cover and no-cover plots ( $P = 0.6783$ ). We present the data as the sum of each form of N collected over the entire period that we monitored (Fig. 7a). Ammonium tended to represent a greater proportion of the total inorganic N collected in the

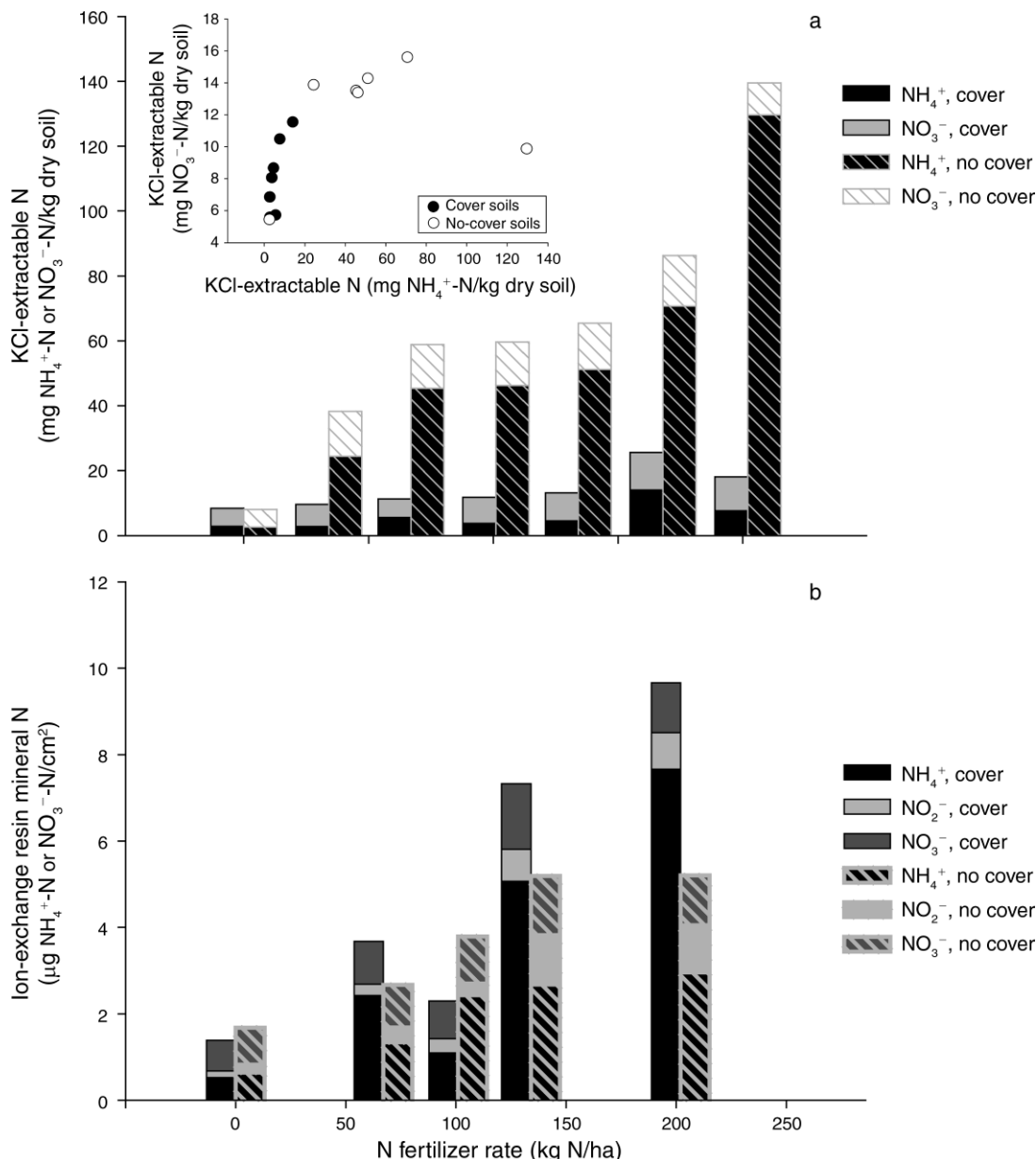


FIG. 6. (a) KCl-extractable ammonium (dark lower portion of each bar) and nitrate (light upper portion of each bar) for soils collected 17 July 2006 at each of the seven N application rates, with (no hatching, left bars in each pair) and without (hatched, right bars in each pair) cover incorporated into the soil. The inset represents ammonium vs. nitrate for all N rates, with and without cover incorporated into the soil. (b) Total mineral N (ammonium + nitrate + nitrate) accumulated on ion-exchange strips deployed for a two-week interval from 5 July to 19 July 2006, the period preceding the soil sampling presented in panel (a).

cover plots relative to the no-cover plots ( $P = 0.0931$ ). At 202 kg N/ha, NO<sub>3</sub><sup>-</sup> represented a greater proportion of the inorganic N in no-cover compared to cover plots (Fig. 7a, b). Nitrite was also present on our resin strips (Fig. 7a). In plots where no N was added, we saw greater N accumulation on ion-exchange resin strips from the no-cover plots early and late in the field season relative to the cover plots, similar N accumulation when maize growth was most rapid in late July and August, and

greater accumulation on resin strips from the cover than the no-cover plots in late August through September (Fig. 7c).

At harvest, accumulation of N in maize plants grown without fertilizer additions provided a final estimate of plant N availability. Cover-cropped soils produced maize plants that contained less N in their aboveground parts: 37 kg N/ha vs. the 49 kg N/ha for the no-cover plots ( $P = 0.00681$ ).

### *N* export as $N_2O$ and $NO_3^-$

Cover crop incorporation into soils did not stimulate N fluxes as  $NO_3^-$  and  $N_2O$ . Under N-deficient (0 kg N/ha) and N-sufficient (101 kg N/ha) conditions,  $N_2O$  and  $NO_3^-$  losses were similar with and without cover crops (Fig. 8a, b, d, e). Under excessive N levels (202 kg N/ha), no-cover plots tended to have greater N losses compared to cover plots (Fig. 8c, f). Nitrous oxide fluxes and  $NO_3^-$  concentrations were higher in plots that had excessive N applied compared to N-deficient and N-sufficient plots regardless of cover treatment (Fig. 8). Nitrous oxide fluxes were higher in 2007 than 2006, potentially due to time of sampling relative to rainfall (gas sampling occurred immediately after rain events in 2007).

## DISCUSSION

### *Immobilization/mineralization and N availability*

We found that cover crop incorporation decreased soil available N early in the growing season, rather than enhancing plant/microbe-available N pools. Incorporating cover crop biomass on 20 May 2006 reduced extractable soil mineral N through 17 July 2006 (Fig. 6) and decreased maize biomass early in the growing season at least until 2 August 2006 (Fig. 3). The most plausible explanation for the reduction in soil N levels and decreased maize biomass on the cover-cropped plots is that the incorporation of high C:N ratio biomass before planting immobilized soil N generated by mineralization early in the growing season, as well as fertilizer N once it was applied in late June. Given that the aboveground wheat biomass was 44% C and assuming that microbial biomass has a C:N ratio of 5:1, up to 92 kg N/ha could have been immobilized, without taking roots into account. In addition, chlorophyll meter readings, one indicator of plant N status, corroborate N deficiency in the maize plants on the cover-cropped plots relative to the no-cover plots on 2 August 2006 and relief of the deficiency by 18 August 2006 in all but the unfertilized plots. In 2007, there were similar quantities of aboveground biomass and N incorporated into the soil for both the cover and no-cover plots because weed growth occurred on the no-cover plots. Early-season maize biomass was similar for both cover and no-cover plots, most likely due to similar quantities of N provided by incorporated biomass. Sanchez and others (2004) found that amount and diversity of biomass in cover/no-cover comparisons was similar when weeds were allowed to grow in no-cover plots for a study adjacent to this study.

Another potential mechanism for biomass reductions on cover-cropped soils is allelopathy. If reductions in crop biomass were due to allelopathy caused by cover crop incorporation, we would expect more N in the cover soils relative to the no-cover soils due to hindered crop uptake. Instead, we saw a decrease in N levels in

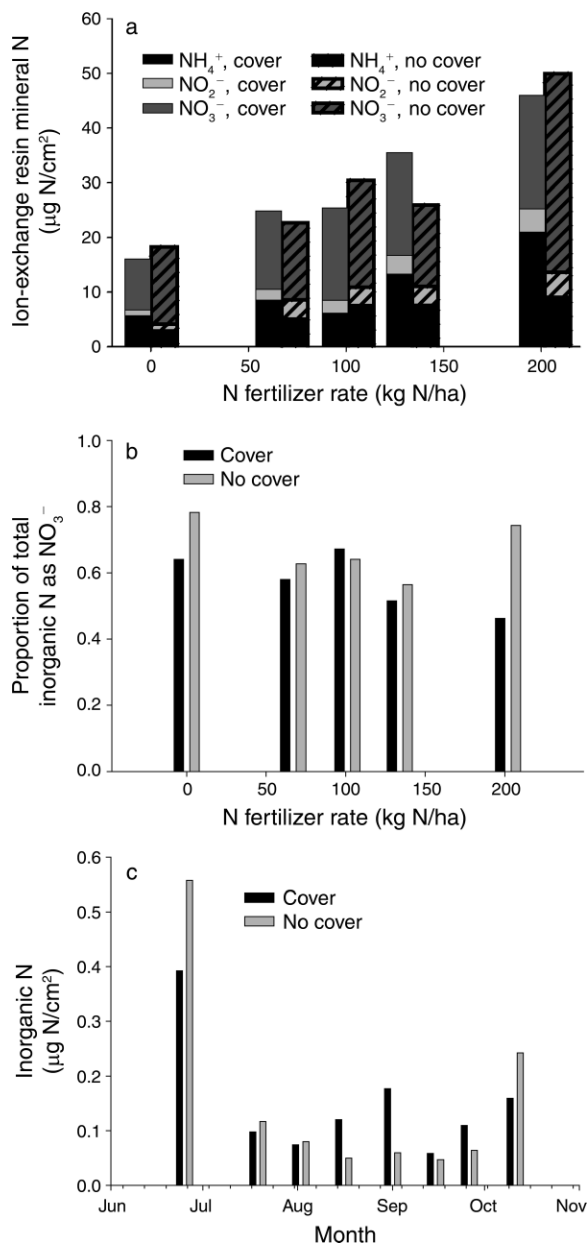


FIG. 7. (a) Total mineral N (ammonium + nitrate + nitrite) accumulated on ion-exchange strips deployed for two-week intervals from 18 June to 11 October 2006. Ammonium is the bottom portion of each bar, nitrite the middle portion, and nitrate the top portion. The solid bars are for plots where cover crop was incorporated, and the hatched bars are for plots that had no cover incorporated. (b) Proportion of total inorganic N accumulated over the 2006 field season that was  $NO_3^-$ . (c) Contrast between total inorganic N collected on resin strips in the cover and no-cover plots with 0 kg N/ha for each two-week deployment in 2006. The difference between the two bars for each date, summed for all measurements made in 2006, equals 0.

cover-cropped soils early in the field season (Fig. 7c), so allelopathy is not likely responsible for the patterns that we saw. The large differences observed between cover and no-cover soil mineral N levels two to three weeks

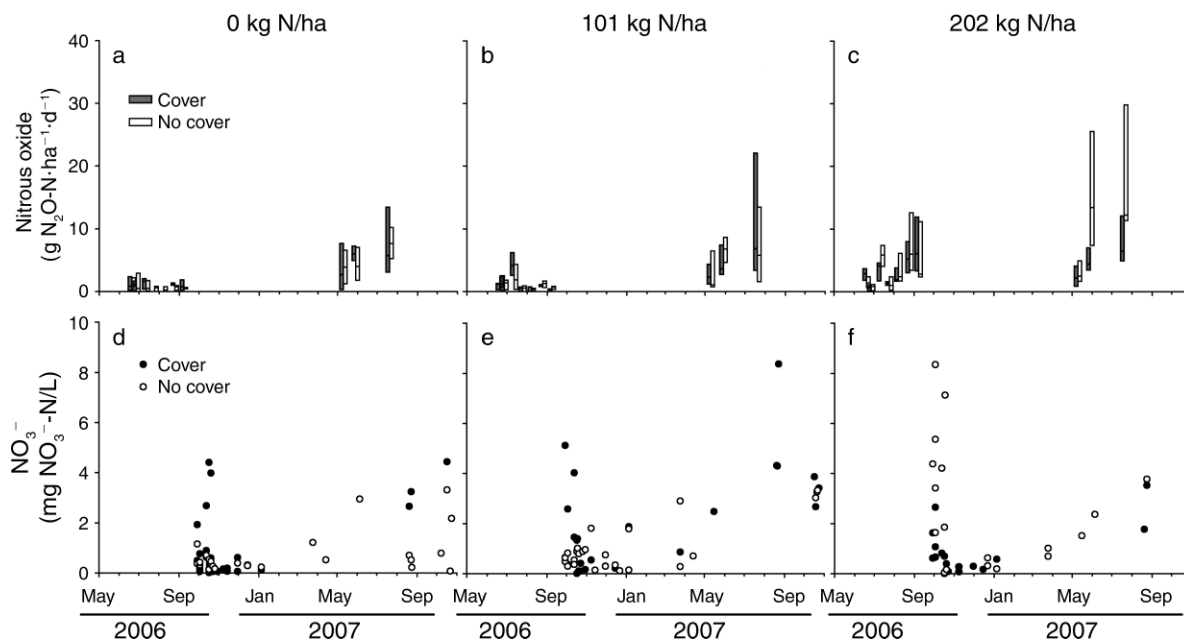


FIG. 8. (a, b, c) Nitrous oxide fluxes and (d, e, f)  $\text{NO}_3^-$  concentration for 2006 and 2007 under three levels of N fertilizer application: deficient (0 kg N/ha), sufficient (101 kg N/ha), and excessive (202 kg N/ha). In panels (a)–(c), the left side (dark box) at each time point represents cover plots, and the right side (white box) represents no-cover plots.

after fertilization further supports immobilization as the most likely mechanism for our observed N deficiencies.

Despite the lower soil N and maize crop biomass early in the growing season for the cover crop plots, late-season N availability and maize biomass at physiological maturity indicate that the incorporated cover crop did not decrease maize yields. Our observation that cover crop amended soils started with lower levels of mineral N, yet mineralized to a greater extent during incubation than non-amended soil ( $24$  vs.  $15 \text{ kg N}\cdot\text{ha}^{-1}\cdot[28 \text{ d}]^{-1}$  for cover and no-cover soils, respectively) supports the potential for an immobilization/release mechanism. The fact that the amount of N available to unfertilized maize plants was greater in no-cover than cover plots in late June, similar in cover and no-cover plots in late July and early August when crop N uptake is greatest, and greater in cover than no-cover soils in August and September, provides additional evidence that N was immobilized early and slowly released later in the growing season (Fig. 7c). Nitrogen accumulation on ion-exchange resins was similar for the cover and no-cover plots over the entire growing season and for all N rates (Fig. 7a). Finally, there was no difference between cover and no-cover maize aboveground biomass or total N in aboveground maize biomass for either 2006 or 2007. We cannot state specifically the source of N taken up later in the field season by maize grown on cover-cropped soils. Late-season N could have been either the N that had been immobilized earlier (from mineralization during spring and fertilizer N), or it could have been

supplied through mineralization of soil organic matter and cover crop biomass.

Immobilization of soil N can be an impediment to winter cover crop adoption by growers because maintenance of adequate soil N during crop growth is required to achieve yield goals. Where cover crops are incorporated into the soil before planting, yields can be maintained by killing cover crops earlier to allow more time for mineralization and by applying fertilizer earlier so that if immobilization does occur, the N stress can be relieved (Crandall et al. 2005). By maintaining soil N levels with starter N at planting, and applying the remainder of the N at four and seven weeks after planting, Andraski and Bundy (2005) have shown increases in maize yields at multiple N rates in their cover-cropped plots compared to no-cover plots. In 2006, the cover crop was killed two weeks before planting and all of the N fertilizer was applied at the six-leaf stage, with no reductions in maize yields in the cover treatment. Our results support the recommendation of Crandall et al. (2005) that when no starter N is applied, yields will be maintained if fertilizer is applied by the six-leaf stage. Monitoring of N availability and N export in this study provides insight into why our yields, and potentially those of others, might be maintained, in situations where N is immobilized.

#### *N losses and N availability*

The hypothesis that magnitude and timing of N losses would be altered with C and N additions to soil pools via cover crop incorporation was not supported. We did not

see greater  $\text{N}_2\text{O}$  or  $\text{NO}_3^-$  fluxes in the cover plots compared to the no-cover plots during the growing season in 2006. Instead, N fluxes were greater in the no-cover plots relative to cover plots that were fertilized at the highest N rates. This is not surprising for 2006, as small quantities of rainfall came at steady intervals throughout the growing season, allowing efficient N use by the crop. We calculated apparent N recoveries that were as high as 90% for some of the lower N rates (Table 3). Estimates of fertilizer N efficiency in row crops range from 30% to 60% (Cassman et al. 2002, Balasubramanian et al. 2004, Krupnik et al. 2004, Nyiraneza and Snapp 2007). For 2006, more N was accumulated in aboveground maize biomass than was applied in N fertilizer for all rates except the 168 and 202 kg N/ha treatments; and therefore, we only expect to see N export from the two highest N rates. Indeed, the 202 kg N/ha treatment, which we chose to represent the highest N rates, exhibited larger exports of  $\text{N}_2\text{O}$  and  $\text{NO}_3^-$  (Fig. 8). Nitrogen accumulation on resin strips, which integrates the effects of soil water content and soil N concentrations, further corroborates the increase in N availability that drove increased fluxes at 202 kg N/ha (Fig. 8).

Past work has shown that the effect of winter cover crops on  $\text{N}_2\text{O}$  emissions is variable, both when they are growing and after they have been killed. Some have found that incorporation of winter wheat before planting had no effect on  $\text{N}_2\text{O}$  emissions, whereas incorporation of other cover crops reduced  $\text{N}_2\text{O}$  fluxes (Baggs et al. 2000). In a no-till maize-soybean rotation, Parkin and Kaspar (2006) saw no difference in  $\text{N}_2\text{O}$  fluxes between plots with and without rye, either when the rye was growing or after it had been killed and the maize crop was growing. In growth-chamber studies, Parkin et al. (2006) found that growing rye plants reduced  $\text{N}_2\text{O}$  fluxes where they had applied 75 or 195 kg N/ha as swine manure when compared to no N additions. In soil core studies, Rosecrance et al. (2000) showed that rye reduced  $\text{N}_2\text{O}$  fluxes when there were growing plants present and evidence of N immobilization after the rye had been killed. For the sandy soils in our field study, the cover crop had the greatest effect on  $\text{N}_2\text{O}$  fluxes when soil N levels were high due to application rates that exceed the uptake capacity of the crop.

Incorporation of a cover crop may reduce nitrification rates. In soils collected on 12 June 2006,  $\text{NO}_3^-$  concentrations in no-cover soils were two times that in cover soils, (11.2 g/kg vs. 5.6 g/kg). For soils collected on 17 July 2006, it appears that nitrifiers became saturated in the no-cover plots where the  $\text{NH}_4^+$  concentrations increased with increasing N addition and  $\text{NO}_3^-$  concentrations did not increase monotonically (Fig. 6). Whereas in the cover soils  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were lower than those in no-cover across all N additions, except at 202 kg N/ha where  $\text{NO}_3^-$  concentrations were similar in both types of soils (Fig. 6). Immobilized N may be slowly fed back to the microbial community at a slow rate in the cover soils. There are two potential

TABLE 3. Maize apparent fertilizer N recovery for cover and no-cover plots.

Applied N rate (kg N/ha)	Cover apparent recovery (%)	No-cover apparent recovery (%)
34	72	93
68	95	88
101	87	82
134	87	67
168	75	59
202	54	50

Note: Recovery was calculated using [(kg N/ha in aboveground biomass for X kg N/ha fertilizer applied) - (kg N/ha in aboveground biomass for 0 kg N/ha fertilizer applied)]/X kg N/ha fertilizer applied.

explanations for why we see less  $\text{NO}_3^-$  in our cover plots compared to our no-cover plots. First, less  $\text{NH}_4^+$  may have been available for nitrifiers due to immobilization of the N into the cover crop that was incorporated. Second, localized anaerobiosis due to  $\text{O}_2$  consumption during microbial decomposition of the cover biomass can hinder nitrifiers. Regardless of mechanism, reduction of nitrification would be a net positive for the agroecosystem, as less  $\text{NO}_3^-$  will leach if less is produced. In addition, less  $\text{NO}_3^-$  would decrease the potential for production of  $\text{N}_2\text{O}$  via denitrification. Previous studies at KBS demonstrated decreases in potential nitrification in soils that received N from compost relative to N applied in fertilizer (Fortuna et al. 2003).

McCracken et al. (1994) saw differences between fallow and winter rye cover in  $\text{NO}_3^-$  leaching of 37.3 and 1.5 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, respectively, in one year of their study and that cover-cropped plots had the lowest  $\text{NO}_3^-$  leaching rates whether the maize crop was growing or the rye. Parkin et al. (2006) found that growing rye plants reduced  $\text{NO}_3^-$  leaching where they had applied 75 or 195 kg N/ha as swine manure in growth-chamber studies. Rosecrance et al. (2000) saw reductions in  $\text{NO}_3^-$  leaching in soil cores with living plants relative to fallow cores, and up to one week after killing the plants. Rasse et al. (2000), in a study of rye cover crop in maize agroecosystems, concluded that an N rate of 101 kg N/ha is environmentally safe because N leaching remained low up to this N rate, regardless of cover crop presence. The maximum amount of N that accumulated in the grain fraction was 101 kg N/ha (data not shown), which suggests that providing N at a rate that offsets the export of N in grain can result in low N losses and maximum yield potential.

#### *N immobilization and yield*

The results from this study suggest that N immobilization may reduce N losses without decreasing maize yields, particularly at high N rates, in two ways. First, by temporarily tying up N that could have been exported as  $\text{N}_2\text{O}$  or  $\text{NO}_3^-$ , we created a biological slow-release fertilizer. Nitrogen provided to the maize plants could

have come from the soil organic-matter pool or immobilized soil or fertilizer N being rereleased. Second, we decreased the concentration of  $\text{NO}_3^-$  at excessive N rates with our cover crop additions, as measured by ion-exchange resins and in soil samples, and therefore reduced the potential for  $\text{NO}_3^-$  leaching. Recent work in vegetable systems has examined the feasibility of applying “immobilizer wastes” to fields to soak up residual fertilizer N and the N mineralized from vegetable residues to prevent leaching of  $\text{NO}_3^-$ , followed by “mobilizer wastes” to generate pulses of mineralization to release N for subsequent crops of *Lolium perenne* L. (ryegrass), *Allium porrum* (leek), and *Lactuca sativa* L. (lettuce) (Chaves et al. 2007). Montemurro et al. (2006) applied N as a combination of olive wastes and mineral N to a maize–barley (*Hordeum vulgare* L.) rotation and found that they attained similar yields, an increase in total soil organic C, and a decrease in mineral N deficit with no difference in N utilization efficiency as compared to strictly adding mineral fertilizer for both crops. Comparison of N cycling in organic and conventional agricultural systems suggests that the accumulation of organic matter in the organic system provides a slow feed of N to the crop (Burger and Jackson 2003).

Current understanding of the N cycle supports the potential to use biological processes in conjunction with energy-intensive fertilizer inputs for a tighter cropping system N cycle (Drinkwater and Snapp 2007). Studies in temperate (Zak et al. 1990, Nadelhoffer et al. 1995, 1999) and tropical (Zimmerman et al. 1995) forests, a riparian zone (McSwiney et al. 2001), and grasslands (Barrett and Burke 2000, 2002) have shown that in some situations, N immobilization can have a significant influence on N availability. In addition, crops, particularly maize, can take a larger proportion of their N from soil pools than from applied fertilizer (Reddy and Reddy 1993, Omay et al. 1998, Cassman et al. 2002, Stevens et al. 2005). It has also been proposed that plants may stimulate mineralization to varying degrees, and as a result, their own N supply (Hamilton and Frank 2001). Controls on whether plants stimulate or inhibit N mineralization/immobilization include plant species, plant developmental stage, and soil N status (Bremer and Kuikman 1997, Fu and Cheng 2002, Cheng et al. 2003). For fields with a history of organic-matter additions at KBS, we have evidence that maize plants may be stimulating their own N supply (Sanchez et al. 2002). By providing a diverse pool of soil organic matter to crops that have the ability to stimulate N mineralization and N at a rate that is sufficient may create the best balance between immobilization and plant stimulated mineralization, and therefore a system that is less likely to “leak” N.

#### CONCLUSIONS

From this study, we suggest that N immobilization has the potential to mediate N losses while maintaining

yields in conventional agroecosystems. In this study, we demonstrate reductions in soil N pools three weeks after fertilization, less N accumulated on resin strips early in the field season, and N-stressed maize plants for much of the growing season in cover plots compared to no-cover plots. Despite decreased N availability in cover plots, slow releases of N in soil pools allowed maize plants in cover plots to recover and attain similar yields to those that were grown in soils without cover crops. At least for the two forms of N export considered,  $\text{N}_2\text{O}$  fluxes and  $\text{NO}_3^-$  leaching, incorporation of a cover crop into the soil may decrease N losses when N rates applied exceed grain N requirements during the early growing season.

The results of this study have several management implications. First, by applying N at rates similar to the amount of N that will be removed in grain at harvest, growers would maintain their fields in N balance. Second, N immobilization due to cover crop incorporation should be considered an N management tool that can retain N in the system and support yields. Finally, incorporating cover crops into conventional agricultural systems do not stimulate N losses, and in fact may tighten the N cycle when N additions are large.

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