

## Nitrogen fertilizer effects on soil carbon balances in Midwestern U.S. agricultural systems

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**Abstract.** A single ecosystem dominates the Midwestern United States, occupying 26 million hectares in five states alone: the corn–soybean agroecosystem [*Zea mays* L.–*Glycine max* (L.) Merr.]. Nitrogen (N) fertilization could influence the soil carbon (C) balance in this system because the corn phase is fertilized in 97–100% of farms, at an average rate of 135 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>. We evaluated the impacts on two major processes that determine the soil C balance, the rates of organic-carbon (OC) inputs and decay, at four levels of N fertilization, 0, 90, 180, and 270 kg/ha, in two long-term experimental sites in Mollisols in Iowa, USA. We compared the corn–soybean system with other experimental cropping systems fertilized with N in the corn phases only: continuous corn for grain; corn–corn–oats (*Avena sativa* L.)–alfalfa (*Medicago sativa* L.); corn–oats–alfalfa–alfalfa; and continuous soybean. In all systems, we estimated long-term OC inputs and decay rates over all phases of the rotations, based on long-term yield data, harvest indices (HI), and root:shoot data. For corn, we measured these two ratios in the four N treatments in a single year in each site; for other crops we used published ratios. Total OC inputs were calculated as aboveground plus belowground net primary production (NPP) minus harvested yield. For corn, measured total OC inputs increased with N fertilization ( $P < 0.05$ , both sites). Belowground NPP, comprising only 6–22% of total corn NPP, was not significantly influenced by N fertilization. When all phases of the crop rotations were evaluated over the long term, OC decay rates increased concomitantly with OC input rates in several systems. Increases in decay rates with N fertilization apparently offset gains in carbon inputs to the soil in such a way that soil C sequestration was virtually nil in 78% of the systems studied, despite up to 48 years of N additions. The quantity of belowground OC inputs was the best predictor of long-term soil C storage. This indicates that, in these systems, in comparison with increased N-fertilizer additions, selection of crops with high belowground NPP is a more effective management practice for increasing soil C sequestration.

**Key words:** agroecosystems; carbon mineralization; corn, oats, alfalfa, and soybean crop rotations; Midwestern U.S. corn–soybean ecosystem; Nashua and Kanawha sites, Iowa, USA; net primary production; nitrogen fertilization; root production; soil carbon sequestration.

### INTRODUCTION

The dominant ecosystem of the Midwestern United States is the corn–soybean (CS) agricultural system, which accounts for 74% of cropland in the region or 25.6 million hectares. In the state of Iowa this system covers 85% of the harvested cropland and 56% of the state's area (USDA-NASS 1997a). On an areal basis, the Corn Belt represents 14% of the principle cropland of the United States (Paustian et al. 1997). Because corn grain yield is increased by the addition of N fertilizer, 97–100% of land cropped to corn is fertilized with N, at an average rate of 135 kg/ha, for a total of 2.1 Gg N added per year in five states alone (Illinois, Iowa, Missouri, Minnesota, and Wisconsin) (USDA-NASS 1997b). Numerous studies in this system have addressed the

effects of N fertilization on soil carbon sequestration (e.g., Khan et al. 2007) and soil quality (e.g., Russell et al. 2006). Lacking, however, is an integrated assessment of the effects of N fertilization on the processes that influence the soil C balance.

The balance of organic carbon (OC) in the soil is determined by rates of OC inputs from net primary production (NPP) of the crops plus any manure additions, and rates of OC decay. In some landscapes, hydrologic losses also play an important role in the soil C balance. On a global basis, however, Van Oost et al. (2007) estimated the C sink for OC deposited by erosion from agricultural systems to be 0.12 Pg C/yr, indicating that, on average, erosion losses comprise a relatively small proportion of OC losses from agricultural systems. Modeled soil-C balances suggest that CO<sub>2</sub> emissions to the atmosphere account for >98% of total C outputs from U.S. croplands, with methane emissions and dissolved OC leaching losses accounting for <1% and <2%, respectively (Li et al. 2003). These data suggest

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that OC decay is the primary pathway of OC loss from soils.

The input and decay rates of OC can be influenced by environmental factors, including temperature, precipitation, and soil type, and by management factors such as tillage and fertilization. We suggest that where only the N-fertilization regime differs, the effect on the soil-C balance could occur by at least three mechanisms. Fertilizer additions could influence NPP and, thus the quantity of OC inputs. Fertilization could alter the partitioning of NPP among plant components above- and belowground, and thereby influence the OC decay rates if decay rates differ with respect to location of decomposition. Finally, N fertilization could alter the crop-tissue chemistry, which would influence its decomposition rate. Long-term studies indicate that initial N concentration drives litter decay rates (Parton et al. 2007). In comparisons among crops, Johnson et al. (2005) also found that initial N influenced decomposition rate.

A better understanding of the effects of N fertilization on the processes that influence the soil C balance can inform management decisions in this era when agroecosystems are increasingly expected to meet multiple needs for food, fiber, biofuels, and soil C sequestration. Many predict that under better management practices, U.S. croplands could sequester C at rates ranging from 60 to 208 Tg C/yr ( $Tg = 10^{12}$  grams) (Lal et al. 1998, Bruce et al. 1999, Allmaras et al. 2000, Paustian et al. 2002, Sperow et al. 2003). Nitrogen fertilization is widely believed to increase soil C sequestration (e.g., Buyanovsky and Wagner 1998), as well as increase yield. To maintain a positive soil C balance, however, N fertilization would have to stimulate rates of OC inputs relatively more than it does OC decay rate, given that decay is the major pathway of OC losses from these systems.

The objectives of this study were to evaluate the effect of long-term N fertilization on the two major processes that control the soil C balance, the production and decay of OC inputs. We also assessed two mechanisms of influence by evaluating the effect of N fertilization on the C:N ratio of corn tissues, and on the partitioning between above- and belowground OC inputs. In an experimental setting, we evaluated three broad hypotheses: (1) organic-C inputs (above- and belowground) do not increase with N fertilization; (2) the C:N ratio of corn tissues does not respond to N fertilization; and (3) organic-C decay rates do not increase with N fertilization.

#### MATERIALS AND METHODS

Our experimental approach involved the evaluation of our three hypotheses (immediately above) in two long-term experimental sites in which soil, climate, slope, tillage, other management practices, and previous history were similar within a cropping system. The CS (corn-soybean) system was the focal point of our study, but we

also estimated organic-carbon (OC) inputs and decay rates in other replicated cropping systems within the experimental matrix at these sites. The other systems included continuous corn for grain (CC), corn-corn-oats-alfalfa (CCOA), corn-oats-alfalfa-alfalfa (COAA), and continuous soybean (SS).

#### Study sites

We conducted these studies at two sites, Kanawha, which is located in north-central Iowa ( $93^{\circ}17'$  E,  $42^{\circ}94'$  N), and Nashua, situated in northeast Iowa ( $92^{\circ}54'$  E,  $42^{\circ}95'$  N), USA. These sites are the longest-running, replicated N-fertilizer experiments in the state of Iowa, having been established in 1954 at Kanawha and 1979 at Nashua. The sites are located within the Iowa State University Northern and Northeast Research and Demonstration Farms, respectively. Mean annual precipitation (MAP) was 806 mm at Kanawha and 847 mm at Nashua over a 53-yr period (Iowa Environmental Mesonet 2004: Clarion and Charles City Stations). Mean annual temperature was  $7.8^{\circ}\text{C}$  at Kanawha and  $8.2^{\circ}\text{C}$  at Nashua. Both sites are situated on soils of the same taxonomic order (Mollisol), but the soils differ, primarily with respect to clay content, so that inherent fertility is higher at Kanawha (Russell et al. 2005). Nashua fine-loamy soils are Typic and Aquic Hapludolls, and Kanawha clay-loam soils are Typic Haplaquolls. Slopes are essentially zero at both sites.

The main tillage at Nashua is chisel plowing to a depth of 23–25 cm in the fall following corn and alfalfa (Russell et al. 2005). At Kanawha, moldboard plowing to a depth of 25 cm is the primary tillage. At both sites, secondary tillage consists of spring disking to a depth of 8–13 cm after soybean, and in plots that had been plowed the preceding fall. At both sites, the cropping systems are tile drained and rain fed.

The experimental design is split-plot randomized block, with cropping system in the main plot, and N fertilization in the subplot. The design at Nashua contains three blocks, and at Kanawha two blocks. In both sites there are four levels of N-fertilizer treatments, 0, 90, 180, and 270 kg/ha, added as granulated urea in all corn phases of the rotation, in the spring immediately before disking. In both sites, the design contains the CC, CS, and CCOA cropping systems, with all phases of the rotation represented in all years. The fourth cropping system differs between the sites: at Kanawha it is COAA, whereas at Nashua it is SS. The experimental unit is the N-fertilization subplot, with a size of  $4.6 \times 15.2$  m at Nashua and  $6.1 \times 12.2$  m at Kanawha. We sampled two transects within each subplot at each site; each transect was the width of the crop row, 0.76 m, and the length of the subplot. Sampling was not conducted within the 1-m-wide buffer zone between contiguous plots. The corn varieties sampled were: Golden Harvest 2390 (non-Bt) at Nashua in 2001; and Golden Harvest 8067 Bt at Kanawha in 2002 (“Bt” indicates this is genetically modified to contain *Bacillus thuringiensis*

genes). The density of corn was the same in all sites:  $8.1 \pm 0.2$  plants/m<sup>2</sup> (mean  $\pm$  SE).

For Nashua, an undisturbed prairie situated 50 km to the north provided a native-vegetation reference point for soil organic carbon (SOC) measurements in the experimental plots. Hayden Prairie is the oldest prairie preserve in Iowa and contains a species-rich assemblage of grasses, other monocots and many species of forbs. The dominant plant species were *Andropogon gerardii* Vitman (big bluestem) and *Helianthus* spp. (sunflower) (Russell et al. 2005). The predominant soil series were similar to those at Nashua. Native prairie no longer exists near Kanawha.

#### *Organic-carbon inputs*

No manure or mulch additions were added at any time, so organic matter (OM) inputs for all crops (corn, soybean, oats, and alfalfa) were calculated as aboveground net primary production (ANPP) plus belowground net primary production (BNPP) minus harvested yield. To place these inputs on a C basis, OC inputs were calculated as the product of OM inputs and C concentration (measured by plant component for each crop). We used the method described by Prince et al. (2001) to estimate NPP from long-term yield data for each N treatment and crop with the cropping systems at our sites. For Kanawha, the yield data are from 1985 to 1998 (Mallarino and Rueber 1999) and for Nashua they are from 1979 to 1998 (Mallarino and Pecinovsky 1999). The allometric relationships used in these calculations included the Harvest Index (HI = harvest index, the ratio of seed yield to aboveground production), for estimating ANPP, and the root:shoot ratio (belowground biomass divided by aboveground biomass at maturity) for estimating BNPP. The strength of this method is that the use of long-term yield data avoids bias that could result from interannual variability in climate, and hence in NPP.

Net primary production and allometric relationships of crops can differ among sites, climate zones, and cultivars. They can also differ over time, as evidenced by Buyanovsky and Wagner's (1998) estimate that the total net annual production of corn and its post-harvest residues have more than tripled since 1950. Root production can vary widely among sites and cultivars (Prince et al. 2001), yet estimates of root:shoot ratios are typically based on a few published ratios (Allmaras et al. 1975, Anderson 1988). To assess the effect of these differences in allometries on estimates of NPP from yield data, Prince et al. (2001) conducted sensitivity analyses, based on published ranges of these ratios, and found the limit of accuracy to be  $\sim 1$  Mg·ha<sup>-1</sup>·yr<sup>-1</sup>. These analyses indicated that interannual fluctuations in NPP would introduce far more bias than variability in the allometric ratios. Thus, we reasoned that estimations of NPP from long-term yield data would introduce the least bias. This method does not address the effect of N-fertilization level on these allometries, however, so we measured

ANPP and BNPP of corn, the only N-fertilized crop, as follows.

*Aboveground NPP of corn.*—We measured aboveground biomass of corn at maturity ( $\sim 20$  weeks, black layer stage) in the CS system under the four N treatments in each of the replicates in a single year per site. Locations for  $0.5 \times 0.76$  m quadrats were randomly selected and framed with PVC tubing. We harvested all of the aboveground biomass from each of the six plots per N treatment within a site, sampling a total of  $\sim 200$  plants within the 48 plots in the two sites. We separated the biomass into the following components: grain, leaves, stalks, husks, cobs, tassels, aboveground prop roots, weeds, and detritus (current crop only). Losses to herbivory were minimal; hence, total aboveground biomass at maturity of this annual crop provided a measure of ANPP.

*Belowground NPP of corn.*—To assess the assumption that root biomass at maturity represented annual root production, we conducted a pilot study at a site in north-central Iowa that contained N treatments of 67, 135, and 202 kg N/ha. We assessed root mortality at three times during the growing season, at ages 3, 12, and 20 weeks. At each sample time, the location of three quadrats ( $0.5 \times 0.76$  m) for each N treatment was randomly selected, the quadrat was framed with PVC tubing, and the entire soil mass was excavated to a depth of 0.5 m. In total,  $\sim 105$  plants were sampled. The entire mass of soil and roots was soaked overnight and roots were separated from soil and detritus by hand. Roots were categorized by status (live or dead), based on morphological characteristics. Samples were dried at 65°C and weighed before calculating the proportion of dead mass. At 3, 12, and 20 weeks, the dead mass was  $0\% \pm 0\%$ ,  $8.7\% \pm 1.0\%$ , and  $6.8\% \pm 0.8\%$  (mean  $\pm$  SE) of total root mass, respectively. Root mortality rates this low at all plant ages indicated that root biomass at maturity provided a reasonable measure of annual root production. Thus, our measurement of the belowground components of corms, fine roots, and coarse roots provided an index of belowground net primary productivity (BNPP); measures of root hair production, root exudates and microbial growth, are not included in this index.

Biomass of fine roots, belowground prop roots, and belowground stem was sampled using a mechanical coring device equipped with an inner plastic sleeve (6.1 cm diameter). We sampled to a depth of 110 cm at four positions: (1) the inter-row; (2) center of row (between plants); (3) center of row (on top of plant); and (4) the midpoint between the row center and the inter-row. Cores were extracted within one week of harvest (at maturity) and refrigerated at 4°C for no longer than one month before processing. Each soil core was first cut into five depth increments, 0–15, 15–30, 30–50, 50–85, and 85–110 cm; each depth increment was processed separately. Initial separation of roots from soil was done using a hydropneumatic elutriation system (mesh size of 410  $\mu$ m) (Smucker et al. 1982). In tests using sewing

threads as an assay for fine roots (mixed with soil), we recovered 100% of initial mass ( $n = 5$  replicates). Roots were separated from detritus by hand and dried at 65°C. Masses were corrected to an ash-free basis. To convert to a mass per unit area basis, values from the four positions were weighted by their area of representation.

*ANPP and BNPP for crops not N-fertilized.*—For soybean, oats, and alfalfa we estimated OM inputs using published allometric relationships. The conversion factors to a basis of megagrams per hectare (Mg/ha) from bushels per acre for corn, soybeans, and oats were 0.06277, 0.06725, and 0.03583, respectively (Prince et al. 2001). For alfalfa, the conversion factor from tons per acre was 2.2412. The ratios for HI were 0.42, 0.52, and 1.00, respectively, and for root:shoot, 0.15, 0.40, and 0.87, respectively, for soybean, oats, and alfalfa (Buyanovsky and Wagner 1997, Prince et al. 2001).

*C and N concentrations.*—Corn tissue samples from the NPP studies were analyzed for C and N. Leaves, stems, and fine roots were sampled in oats, soybeans, and alfalfa at both sites in August 2002, using similar protocols as for corn. All plant samples were dried at 65°C, ground to a fine powder, and analyzed for C and N by dry combustion using a Carlo-Erba NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, New Jersey, USA).

#### Organic-C decay rates

We evaluated the effect of N fertilization on OC decay rate two ways. (1) We estimated OC decay rates in all cropping systems by a mass-balance approach. (2) In the CS system at Nashua we also used an isotopic mixing model to assess decay rates of the C<sub>3</sub>- and C<sub>4</sub>-derived C inputs to the system.

*Mass balance.*—Organic-C decay rates and turnover times were calculated from  $k_t = I/C_t$ , where  $C_t$  is soil C at time  $t$  (Mg C/ha),  $I$  is OC inputs (Mg C·ha<sup>-1</sup>·yr<sup>-1</sup>), and  $k_t$  is the decay rate (yr<sup>-1</sup>; Olson 1963, Paustian et al. 1997). Organic-C inputs were estimated from long-term yield data, as described above, and represent annual inputs averaged over the long term, over all phases of the rotation. Soil OC was measured as described below (see *Soil organic C*). Evidence of overland flow was not observed in these sites, so losses of soil by erosion were negligible. Total SOC stocks had not changed significantly over the last 12 years (Russell et al. 2005) in all but two of nine systems for which time-series data exist. The two significant changes are minuscule relative to the magnitude of the SOC stocks (Russell et al. 2005); thus, even the significant changes had a negligible influence on the calculations of the mass C balance.

*Isotopic mixing model.*—To gain more insight into the decay dynamics, we used an isotopic mixing model to evaluate the effect of N fertilization on humification rates, decay rates, and turnover times of the two SOC pools in the CS system, as estimated from natural δ<sup>13</sup>C abundance (Huggins et al. 1998). Following Jenkinson (1988), decomposition is conceptualized as operating

over relatively short or long time periods; this conceptualization corresponds to the “fast” and “slow” pools of OC, both of which follow first-order kinetics (Jenkinson and Raynor 1977). By this model (Huggins et al. 1998), annual C inputs enter the fast pool, in which labile C is rapidly decomposed, and a fraction,  $h$ , enters the slow pool. Decay rates,  $k$ , are distinguished for the fast and slow pools. To determine decay rates by this model, we measured C isotope ratios in corn and soybean tissues collected as described above, and in soil (0–15 cm) in the C<sub>4</sub> system (CC), the C<sub>3</sub> system (SS), the C<sub>4</sub>–C<sub>3</sub> rotation (CS), and soil in the nearby native prairie. Soil collected in the 2002 sampling (see *Soil organic C*, below) was analyzed for δ<sup>13</sup>C. Only the Nashua site contained all three cropping systems and hence was suitable for this study. The C isotope ratios were expressed as δ<sup>13</sup>C values:

$$\delta^{13}\text{C}(\text{‰}) = [(R_{\text{sam}}/R_{\text{std}}) - 1] \times 10^3$$

where  $R_{\text{sam}} = {}^{13}\text{C}/{}^{12}\text{C}$  ratio for the sample, and  $R_{\text{std}} = {}^{13}\text{C}/{}^{12}\text{C}$  ratio of the working standard, Pee Dee Belemnite. Partitioning of SOC into soybean (C<sub>3</sub>) and corn (C<sub>4</sub>) sources followed

$$\delta_m = f\delta_a + (1 - f)\delta_b$$

where  $\delta_m = \delta^{13}\text{C}$  of SOC from a mixture of C<sub>3</sub> and C<sub>4</sub> sources,  $f$  = fraction of OC from the C<sub>4</sub> source,  $\delta_a = \delta^{13}\text{C}$  from the C<sub>4</sub> source,  $(1 - f)$  = fraction of OC from the C<sub>3</sub> source, and  $\delta_b = \delta^{13}\text{C}$  from C<sub>3</sub> source.

Prior to 1979, the onset of this experiment, the Nashua site had been cultivated in continuous corn. Baseline soil samples did not exist for determination of initial δ<sup>13</sup>C values for this site; given the site history, values of the CC system at the 90 kg/ha N treatment most closely approximated “initial” values. Detailed calculations of rates of humification and decay of fast and slow pools, and turnover times for C<sub>3</sub>- and C<sub>4</sub>-derived C are described by Huggins et al. (1998).

*Soil organic C.*—Soil was sampled in all N treatments in the corn phase of all cropping systems at Nashua and Kanawha in 2002 during a single postharvest sampling (in October) prior to fall tillage. Previous SOC measurements at monthly intervals indicated that sampling at this time introduced the least amount of residual error in the measurements (Russell et al. 2005). Each 1-m-deep soil core was divided into six depth increments, 0–5, 5–15, 15–30, 30–50, 50–75, and 75–100 cm. For all soil sampling, six 4.1-cm-diameter soil cores were taken within each plot, at two points randomly selected within each of the three positions, in the center of the row, in the inter-row, and at the midpoint between those two points. The subsamples within a plot were bulked by depth increment. All samples were air-dried, roots were removed, rock masses and volumes were determined, and soil was passed through a 2-mm sieve. Subsamples were dried at 105°C to determine conversion factors to a 105°C dry-mass basis.

Total soil-concentrations were analyzed by dry combustion, using the Carlo-Erba elemental analyzer

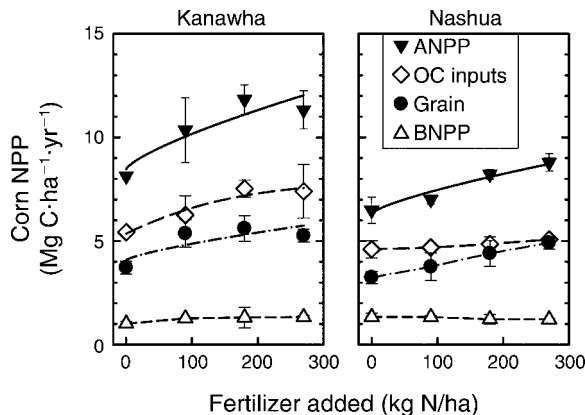


FIG. 1. Response of net primary production (NPP) of corn to N fertilization in two experimental sites in Iowa, USA. Aboveground NPP (ANPP) plus belowground NPP (BNPP) minus harvested grain equals organic-carbon (OC) inputs. Values are medians. Curves represent fitted models.

described above (see *C and N concentration*). Total SOC was calculated as the difference between total soil C and soil inorganic C, which was measured by the modified pressure-calimeter method (Sherrod et al. 2002). Nashua soils did not contain measurable carbonates; at Kanawha the surface (0–15 cm) layer, the focus of this study, was low in carbonates, with soil inorganic carbon only 0.8% of the total soil C. Total SOC storage was calculated as the product of bulk density (BD), soil thickness, and soil C concentration. To compare SOC storage among treatments, all data were expressed on an equivalent-mass basis by layer, as recommended for this type of study (Gregorich et al. 1996, Ellert et al. 2001, 2002, Paul et al. 2001).

#### Statistical calculations

To analyze the effect of N-fertilizer additions, we fit response curves to the OC input and decay response variables, as is recommended for analyzing quantitative response patterns (Mead 1988, Nelson and Rawlings 1988). We tested several mathematical models for their applicability to these data using the SAS system's NLIN procedure (SAS Institute 1999; NLIN procedure *available online*),<sup>4</sup> and chose a model that performed consistently well among all variables for which this model was applied. The Akaike information criterion (AIC), approximate  $R^2$  values, and  $P$  statistics were used to compare a power model with at least one alternative for the each data set. The best-performing power model was a two-parameter form of  $y = c_0 + c_1 \cdot x^{0.75}$ . The AIC represents a log-likelihood value that has been penalized for the number of parameters estimated (Littell et al. 1996). Generally, the AIC is consistent with other performance measures. We used the AIC for small samples sizes ( $AIC_c$ ), with  $AIC_c = AIC + 2p(p + 1)/(n -$

<sup>4</sup> (<http://www2.stat.unibro.it/ManualiSas/stat/chap45.pdf>)

$p - 1$ ), where  $p$  is the number of parameters and  $n$  is the number of observations (Hurvich and Tsai 1995). For the corn NPP study in the CS system, we applied these models to the median value across replicates for each N level because medians are robust, i.e., resistant to outliers. In most cases the mean and median were very close. Two-sided  $t$  tests were applied to test whether soil OC balances differed from zero in the N-fertilized treatments (Snedecor and Cochran 1989). Correlation analysis (Pearson) was applied to evaluate relationships between the location of OC inputs and SOC stocks across all cropping systems and N treatments. In reporting the results of the analyses, we describe trends where  $P$  values were  $\leq 0.15$  and declare them significant where  $P \leq 0.05$ .

## RESULTS

### Organic-C inputs

*Corn only.*—The measured ANPP (aboveground net primary production) of corn in the corn–soybean (CS) system increased with N-fertilizer addition, significantly at Nashua ( $P = 0.03$ ), but not at Kanawha ( $P = 0.07$ ) (Fig. 1; Appendices A and B). Belowground NPP increased with N fertilization at Kanawha and decreased at Nashua, but neither trend was significant ( $P = 0.07$  and  $0.11$ , respectively). Across all N treatments, BNPP (belowground net primary production) comprised 6–22% of total NPP. Thus, given the relatively low BNPP, ANPP dominated total organic-C inputs of corn, which increased significantly with N addition ( $P < 0.05$ , both sites). Grain yield increased with N fertilization only at Nashua ( $P = 0.01$ ) (Fig. 1, Appendix A and B). With N fertilization, corn HI (harvest index; the ratio of seed yield to aboveground production) increased ( $P = 0.01$ ) and root:shoot (belowground biomass divided by aboveground biomass at maturity) decreased ( $P = 0.02$ ) at Nashua (Appendix A). At Kanawha, these ratios did not differ significantly with N fertilization, but the HI values of 0.45–0.51 and root:shoot ratios of 0.11–0.12 across N treatments (Appendix A) were low in comparison with reported values (Prince et al. 2001, Johnson et al. 2006). Thus, we applied our measured allometries that were specific for our sites and N-fertilization levels to calculate organic-carbon (OC) inputs from long-term corn-yield data.

Total corn NPP measured directly in a single year in the CS system at Kanawha ranged from 20 to 28  $\text{Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  (biomass) across N fertilization treatments (Appendix A), higher than that estimated from the long term yield data, 16–24  $\text{Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  (Table 1a). At Nashua, measured total corn NPP in a single year was 18–22  $\text{Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  across N treatments (Appendix A), in comparison with 16–20  $\text{Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  estimated from the long-term yield data (Table 1b).

*All crop phases.*—In comparisons of estimated organic-matter (OM) inputs among crops within a cropping system, corn dominated the inputs in all N treatments in all cropping systems (Table 1a, b). In comparisons of

TABLE 1. Total net primary production (NPP, above- and belowground) and organic-matter (OM) inputs under N-fertilization treatments in two experimental sites in Iowa, USA.

Cropping system	Crop	Total NPP (Mg·ha <sup>-1</sup> ·yr <sup>-1</sup> ), by fertilizer treatment				OM inputs (Mg·ha <sup>-1</sup> ·yr <sup>-1</sup> ), by fertilizer treatment			
		0 kg N/ha	90 kg N/ha	180 kg N/ha	270 kg N/ha	0 kg N/ha	90 kg N/ha	180 kg N/ha	270 kg N/ha
a) Kanawha experimental site									
CS	C	15.6	18.9	23.2	24.3	9.4	10.2	13.4	14.1
	S	7.7	7.8	7.9	7.7	4.9	5.0	5.0	4.9
	Average	11.7	13.4	15.6	16.0	7.1	7.6	9.2	9.5
CC	C	8.4	14.7	19.8	21.9	5.0	8.0	11.4	12.7
	CCOA	22.7	20.9	23.1	23.7	13.6	11.3	13.3	13.8
CCOA	C2	15.5	18.1	22.2	23.4	9.3	9.8	12.8	13.6
	O	5.1	6.2	6.1	6.3	3.2	3.9	3.8	4.0
	A	14.7	15.5	15.1	15.1	6.8	7.2	7.0	7.0
	Average	14.5	15.2	16.6	17.1	8.3	8.0	9.2	9.6
	COAA	24.0	20.8	23.7	23.3	13.6	11.3	13.3	13.8
	O	6.6	6.5	6.8	6.6	9.3	9.8	12.8	13.6
COAA	A1	14.7	13.8	14.7	13.8	3.2	3.9	3.8	4.0
	A2	15.5	14.7	15.5	15.5	6.8	7.2	7.0	7.0
	Average	15.2	13.9	15.2	14.8	8.3	8.0	9.2	9.6
b) Nashua experimental site									
CS	C	16.1	20.3	19.6	19.6	9.9	11.5	10.3	10.1
	S	8.0	8.2	8.1	8.0	5.1	5.2	5.2	5.1
	Average	12.0	14.3	13.9	13.8	7.5	8.4	7.7	7.6
CC	C	8.9	15.4	16.9	17.6	5.5	8.7	8.9	9.1
	CCOA	19.6	20.8	19.8	19.6	12.0	11.8	10.4	10.1
CCOA	C2	12.0	16.8	18.1	18.4	7.3	9.5	9.5	9.5
	O	5.5	5.8	6.3	6.7	3.4	3.7	4.0	4.2
	A	15.9	16.3	15.9	15.5	7.4	7.6	7.4	7.2
	Average	13.2	14.9	15.0	15.0	7.5	8.2	7.8	7.8
	SS								
	S	6.9				4.4			

Notes: Units are megagrams of biomass. Cropping system rotations include: corn for grain (C), soybean (S), oats (O), and alfalfa (A). Nitrogen treatments were applied only in the corn phase of the rotation. Values are based on average yields over the period 1985–1998 for Kanawha and 1979–1998 for Nashua.

inputs on a C basis, averaged across all phases of the rotation, the continuous corn for grain (CC) and CS systems were most responsive, with OC inputs increasing significantly with N fertilization in the CS system at Nashua and Kanawha, and in the CC system at Kanawha ( $P < 0.05$ , 0.05, and 0.01, respectively), with a similar trend in the CC at Nashua ( $P = 0.11$ ) (Table 2, Appendix B). In contrast, OC inputs did not increase significantly with N fertilization at either site in the CCOA (corn–corn–oats–alfalfa) or COAA (corn–oats–alfalfa–alfalfa) systems, although there was a weak trend in the CCOA at Kanawha ( $P = 0.15$ ).

#### Corn C:N, organic-carbon decay

Corn C:N ratios declined with N fertilization for stalks, leaves, grain, fine roots, husks and cobs at both sites (Fig. 2, Appendix B). This trend was significant for all plant components except fine roots at Kanawha ( $P = 0.09$ ) and husks and cobs at Nashua ( $P = 0.08$ ). Consistent with this finding, the OC decay rate, as calculated by mass balance, increased significantly with N fertilization in the CC, CS, and CCOA systems at Kanawha ( $P < 0.01$ , 0.05, and 0.02, respectively) (Table 2, S2). Although not significant, the trend was similar at Nashua in the CC ( $P = 0.15$ ) and CS ( $P = 0.07$ ), but not in the CCOA ( $P = 0.95$ ) system.

By the isotopic mixing model, the estimated proportions of SOC derived from C<sub>4</sub> sources at Nashua were 90%, 90%, 88%, and 87% in the 0 kg/ha, 90 kg/ha, 180 kg/ha, and 270 kg/ha N treatments, respectively, in the CS system ( $\delta^{13}\text{C}$  values are in Appendix C). In comparison, in the CC and SS (continuous soybean) systems, C<sub>4</sub> sources accounted for 93% and 77%, respectively, of the soil organic carbon (SOC). Humification rates of C<sub>4</sub>-derived C increased with N fertilization, but this trend was not significant ( $P = 0.08$ ) (Table 3, Appendix B). Decay rates of the fast pool declined with N fertilization, although not significantly ( $P = 0.10$ ). Decay rates of the slow pool of C<sub>4</sub>-derived C did not increase significantly ( $P = 0.16$ ). The trends were the opposite for the C<sub>3</sub>-derived C in that decay rates of the fast pool increased significantly with N fertilization ( $P = 0.04$ ) and humification rates decreased significantly ( $P = 0.05$ ) (Table 3, Appendix B). The slow-pool decay rate of C<sub>3</sub>-derived C declined with N fertilization, although this trend was not significant ( $P = 0.09$ ).

#### DISCUSSION

Nitrogen fertilization has the potential to stimulate or depress either the rate of organic-carbon (OC) inputs or the decay rate, or it may have no effect on these two rates. There are many possible combinations of effects

TABLE 2. The soil carbon balance under four N-fertilization treatments in two experimental sites in Iowa, USA.

Cropping system	Fertilizer treatment (kg N/ha)	Kanawha				Nashua			
		OC inputs (Mg C·ha <sup>-1</sup> ·yr <sup>-1</sup> )	Decay rate (yr <sup>-1</sup> )	Turnover time (yr <sup>-1</sup> )	SOC† (Mg C/yr)	OC inputs (Mg C·ha <sup>-1</sup> ·yr <sup>-1</sup> )	Decay rate (yr <sup>-1</sup> )	Turnover time (yr <sup>-1</sup> )	SOC (Mg C/yr)
CS	0	3.21	0.070	14.3	46.0	3.35	0.091	10.9	31.4
	90	3.42	0.074	13.5	46.3	3.77	0.106	9.5	34.1
	180	4.14	0.086	11.6	48.0	3.48	0.117	8.5	32.8
	270	4.28	0.098	10.3	43.9	3.42	0.114	8.8	34.4
CC	0	2.25	0.053	18.8	42.4	2.45	0.070	14.3	35.1 a
	90	3.58	0.078	12.9	46.2	3.92	0.104	9.6	37.8
	180	5.14	0.100	10.0	51.3	4.00	0.104	9.6	38.4
	270	5.73	0.120	8.3	47.8	4.08	0.103	9.7	39.5 b
CCOA	0	3.71	0.066	15.2	56.5	3.39	0.087	11.5	39.0
	90	3.62	0.072	14.0	50.7	3.66	0.094	10.6	38.8
	180	4.16	0.077	13.0	54.2	3.52	0.091	10.9	38.5
	270	4.32	0.078	12.9	55.5	3.49	0.086	11.6	40.5
COAA	0	3.67	0.074	13.6	49.8				
	90	3.21	0.055	18.1	58.2				
	180	3.59	0.067	14.9	53.4				
	270	3.63	0.065	15.4	55.9				
SS	0					1.97	0.088	11.4	22.4

Note: Cropping systems contain corn for grain (C), soybean (S), oats (O), and alfalfa (A).

† Data are from Russell et al. (2005). Different letters denote significant differences ( $P < 0.05$ ) in SOC stocks between N treatments within a cropping system.

on these two rates, but it is the balance between the inputs and outputs that determines the quantity of soil organic carbon (SOC) storage. We evaluated the relative effects of N fertilization on the rates of OC inputs and decay in our two long-term experimental sites in Iowa, USA, and assessed these results in light of SOC data, to evaluate the long-term effect of N fertilization on the soil C balance.

#### Net primary production (NPP) and organic-C inputs

Interannual variability in NPP can be quite high in cropping systems, as a result of changes over time in management, weather, and cultivars, and can therefore introduce bias into NPP studies. By using long-term yield data to estimate NPP, we avoided this possible bias. However, changes in cultivars over time could have introduced inaccuracy because the allometric relationships used to estimate NPP can differ among cultivars (e.g., Johnson et al. 2006). Across all cropping systems, the long-term estimated NPP averaged 18 Mg·ha<sup>-1</sup>·yr<sup>-1</sup> at our sites. The limit of accuracy for this estimation method is ~1 Mg·ha<sup>-1</sup>·yr<sup>-1</sup> (Prince et al. 2001), which represents ~6% of the total NPP at our sites. In our study, the accuracy of this method is probably higher because we measured the corn allometric relationships specific for these sites and N-fertilization treatments. Nevertheless, our measured allometries do not account for changes in plant partitioning over time. In their sensitivity analyses, Prince et al. (2001) compared various sources of variability in terms of effects on NPP estimates, and found the effect of changing allometries to be relatively small compared to changes

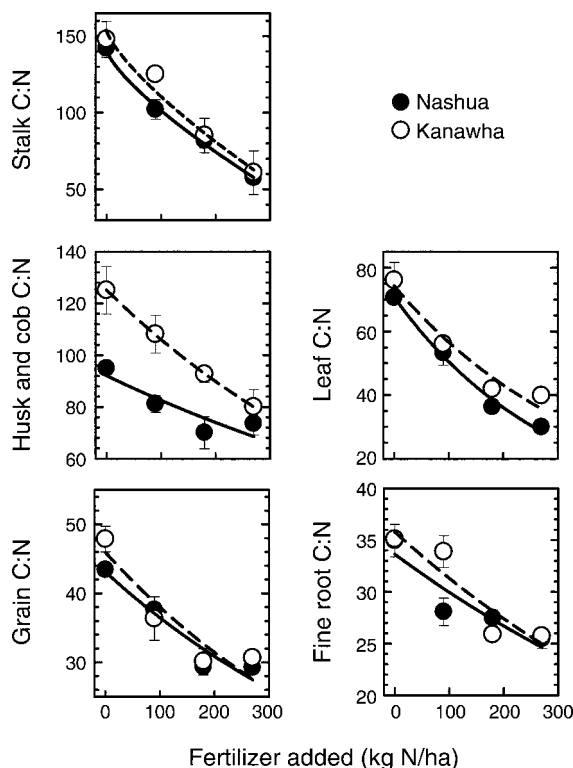


FIG. 2. Effect of N-fertilizer additions on tissue C:N ratios in corn in a corn-soybean rotation. Curves represent fitted models for two long-term sites: Kanawha (dashed) and Nashua (solid) in Iowa, USA.

TABLE 3. Humification rate ( $h$ ), decay rate ( $k$ ), turnover time ( $t_{\text{turn}}$ ), half-life ( $t_{1/2}$ ), and equilibrium levels ( $C_E$ ) of organic matter in a corn–soybean cropping system at the experimental site in Nashua, Iowa, USA, under four N-fertilization treatments.

N-fertilization treatment (kg/ha)	Carbon pool	Carbon derived from C <sub>4</sub> plants					Carbon derived from C <sub>3</sub> plants				
		$h$ (yr <sup>-1</sup> )	$k$ (yr <sup>-1</sup> )	$t_{\text{turn}}$ (yr <sup>-1</sup> )	$t_{1/2}$ (yr <sup>-1</sup> )	$C_E$ (Mg/ha)	$h$ (yr <sup>-1</sup> )	$k$ (yr <sup>-1</sup> )	$t_{\text{turn}}$ (yr <sup>-1</sup> )	$t_{1/2}$ (yr <sup>-1</sup> )	$C_E$ (Mg/ha)
0	Fast	0.174	0.826	1.2	0.8	n.a.	0.654	0.346	2.89	2.00	n.a.
	Slow	n.a.	0.024	41.3	28.6	31.9	n.a.	0.150	6.67	4.62	18.5
90	Fast	0.307	0.693	1.4	1.0	n.a.	0.594	0.406	2.46	1.70	n.a.
	Slow	n.a.	0.050	20.1	14.0	32.1	n.a.	0.108	9.30	6.44	23.5
180	Fast	0.381	0.619	1.6	1.1	n.a.	0.548	0.452	2.21	1.53	n.a.
	Slow	n.a.	0.054	18.5	12.8	32.6	n.a.	0.089	11.28	7.82	26.3
270	Fast	0.351	0.649	1.5	1.0	n.a.	0.552	0.448	2.23	1.55	n.a.
	Slow	n.a.	0.049	20.4	14.1	32.5	n.a.	0.095	10.48	7.30	24.6

Notes: Data are means from the 0–15 cm soil-depth interval for a corn–soybean cropping system, as determined by an isotopic mixing model. Decomposition in the “fast” and “slow” pools occurs on annual and decadal bases, respectively; n.a. = not applicable.

in climate and management. During our single-year NPP study at Nashua during a low-rainfall year, average corn grain yield was 9% lower than the long-term average. At Kanawha, rainfall conditions were optimal during the corn NPP study, and grain yield was 15% higher than the long-term average. This indicates that the long-term yield data do appear to provide more unbiased estimates of long-term organic-matter (OM) inputs than the short-term direct measurements in a single year.

The rates of NPP that we estimated in these systems represent some of the highest NPP reported for temperate-zone croplands (Whittaker and Likens 1975). This is consistent with other analyses that identified a zone of high NPP extending across Iowa, northern Illinois, and Indiana, USA (Prince et al. 2001). Although climate and management contribute importantly to this high NPP, we suggest that the smectitic-clay-rich Mollisols in this region have a nutrient-supply and water-retention capacity that is ideally suited for corn growth. The estimated NPP of our cropping systems is equal to that of temperate forests, nearly double that estimated for natural prairie (Kicklighter et al. 1999), and higher than other reported responses of corn to N fertilization (Huggins and Fuchs 1997). This suggests that these systems have an enormous potential for soil C sequestration.

One obvious factor that could influence this potential, other than management, is the proportion of NPP that is exported from croplands during harvest. Our data indicate that 38–49% of the total NPP was removed from these systems. In the N-fertilized treatments, the corn–soybean (CS) system tended to have the lowest total NPP of the cropping systems studied, owing to the relatively lower NPP in the soybean phase. This translated into lower OC inputs in this system that dominates the Midwestern United States. In comparison with the continuous corn for grain (CC) cropping system, OM inputs were lower in the CS system by

0.4–3.2 Mg/ha (biomass) across N treatments at Kanawha, and 0.3–1.4 Mg/ha at Nashua.

#### Organic-C decay rates

The other obvious factor that influences the potential for sequestration is the decay rate. Broad-scale studies of the controls over decomposition indicate that tissues with lower C:N decompose relatively faster (Parton et al. 2007). In our study, corn tissue C:N declined with N fertilization (Fig. 2), suggesting that decay rates of corn OM inputs would increase with added N. Soils contain a variety of organic matter substrates, however, so that the decay of this medium is complex, with different SOC fractions responding differently. As a result, no single method yields results that are easy to interpret. We used two independent methods, and evaluated results in light of the SOC sequestration data that were available.

The trends in decay rate as determined by the two different methods are consistent with predictions based on corn tissue C:N data. That is, N fertilization stimulated decay rates significantly in the CC, CS and CCOA (corn–corn–oats–alfalfa) systems at Kanawha, as determined by the mass balance (Table 2). At Nashua, decay rates followed the same trends for the CC and CS systems, but were not significant. The decay-rate trends are also consistent with soil C-sequestration measurements that exist for these sites, as discussed below.

The isotopic mixing model results indicated that with N fertilization the humification rate of C<sub>4</sub>-derived C tended to increase, whereas the rate declined significantly for C<sub>3</sub>-derived C (Table 3). In litter-decomposition studies in natural systems, Ågren et al. (2001) found that N fertilization resulted in more rapid formation of recalcitrant material, and proposed increased decomposer efficiency as the mechanism. With respect to effects on the fast pool of OC, which turns over on an annual basis, our results indicated that the decay rate of C<sub>4</sub>-derived C tended to decline with N fertilization (Table 3). In contrast, decay rates of the slow pool, with



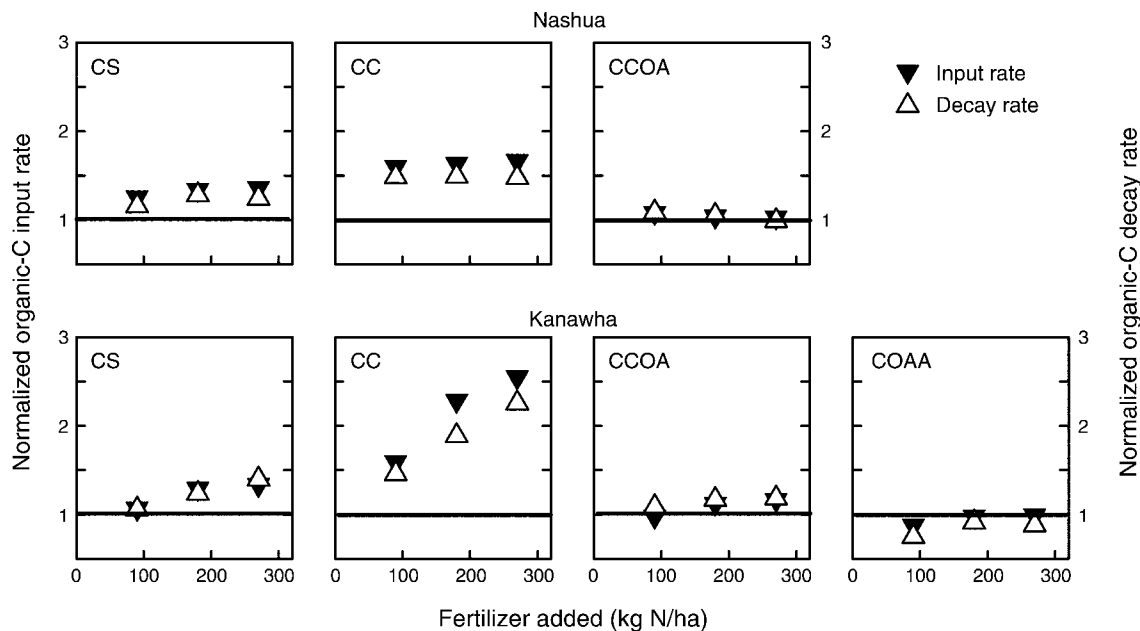


FIG. 3. Effect of N-fertilizer additions on organic-C input and decay rates in cropping systems at two sites in Iowa, USA. Data are based on long-term inputs, averaged over all phases of the rotations, and then normalized by dividing by the value for the unfertilized treatment. The normalized unfertilized treatment is denoted by a line. The crop rotations are: CS, corn-soybean; CC, continuous corn for grain; CCOA, corn-corn-oats-alfalfa; and COAA, corn-oats-alfalfa-alfalfa.

multi-decadal turnover times, tended to increase with N fertilization for the  $C_4$ -derived C. The trends were the opposite for the  $C_3$ -derived C, perhaps owing to higher decomposability of soybean leaves. Other studies in natural alpine-tundra systems found similarly complex effects of N fertilization on the decay dynamics of SOC pools (Neff et al. 2002). In our study, however, it was apparent that inputs of  $C_4$ -derived C dominated dynamics in the CS system because corn OC inputs were approximately double those of soybean (Table 1). The N-fertilization effects on tissue C:N and decay rates as determined by mass balance were also consistent with the effects on slow-pool dynamics of  $C_4$ -derived C, further indicating that this pool dominates SOC dynamics.

The turnover times for the slow pools in this study are appreciably faster than other published values for a site in southwestern Minnesota, USA (Huggins et al. 1998). Our data are for the 0–15 cm soil layer, whereas the Minnesota study concerned the 0–30 cm layer. Also, the Iowa site has a mean annual temperature that is 2°C higher and a mean annual precipitation that is 215 mm greater than the Minnesota site; the warmer, wetter climate in Iowa should promote decay. At Nashua, the equilibrium ( $C_E$ ) values for  $C_4$ -derived soil C (Table 3) were within 3% of the current measured levels of total SOC (Table 2), indicating that soil C stocks are no longer declining significantly. Independent evidence indicates that this is the case. Of the systems for which we have soil C-sequestration data at this site, none had a

significant decrease in SOC between 1996 and 2002 (Russell et al. 2005).

#### Soil organic-carbon balance

To facilitate comparisons across cropping systems regarding the effect of N fertilization on the soil C balance (Table 2), values in the fertilized treatments were normalized by dividing by the control (“0-N” treatment) value within each cropping system (Fig. 3). Nitrogen fertilization resulted in a positive soil C balance, i.e., OC inputs minus decay losses were greater than zero, in the CC system ( $P = 0.07$  at Kanawha,  $P = 0.02$  at Nashua) and the COAA system at Kanawha ( $P = 0.020$ ). The balance between OC inputs and decay did not differ significantly from zero in the other cropping systems. These results are fairly consistent with independent measures of soil C sequestration: SOC storage increased measurably in the N-fertilized CC and COAA systems at Kanawha ( $P = 0.041$  and  $0.002$ , respectively) (Russell et al. 2005). In the other cropping systems at Kanawha and all of the systems at Nashua, however, there were no significant changes in SOC storage with N fertilization over a 12-year period (Russell et al. 2005), indicating that the effect of added OC inputs with N fertilization was essentially cancelled by increased decomposition. These findings are consistent with other studies in that N fertilization has been found to stimulate soil C sequestration in some agroecosystems (Gregorich et al. 1996, Halvorson et al. 1999, Liebig et al. 2002), but not in others (Huggins and Fuchs 1997, Paustian et al. 1997, Halvorson et al. 2002, Leggett and Kelting 2006, Khan

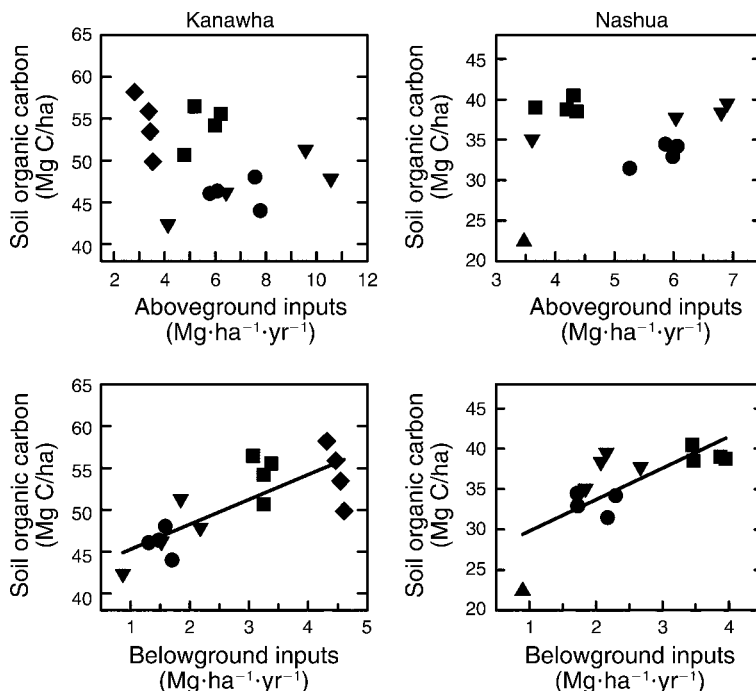


FIG. 4. Relationships between soil organic carbon (0–15 cm), and above- and belowground organic-matter inputs in two long-term sites in Iowa, USA, for different cropping systems: corn-soybean, CS (●); continuous corn for grain, CC (▼); continuous soybean, CS (▲); corn-corn-oats-alfalfa, CCOA (■); and corn-oats-alfalfa-alfalfa, COAA (◆).

et al. 2007). Allmaras et al. (2004) found that although N fertilization stimulated corn stover inputs by 20%, corn-derived SOC increased by only 1.9 Mg C/ha. Regional-level studies have highlighted the variability in predicted sequestration rates, owing to soil taxonomic group, management, and antecedent soil C (Tan and Lal 2005), and certainly these factors could explain the differences in results among sites.

In our experimental setting, however, soil, climate, and management factors were as similar as possible within sites. Also, the relationships between OC input and decomposition rates do not explain why soil OC storage was higher in the CCOA and COAA (corn-oats-alfalfa-alfalfa) systems, relative to the CC and CS systems (Russell et al. 2005). Some other factor must be influencing soil C sequestration. Across all systems, SOC storage was significantly correlated with the quantity of belowground OM inputs (Fig. 4;  $P < 0.01$ , both sites). In contrast, SOC was not correlated with the quantity of aboveground inputs ( $P = 0.45$ , Nashua;  $P = 0.55$ , Kanawha). Similar results have been reported from a variety of sites, by various methods. Balesdent and Balabane (1996) found that corn root-derived SOC contributed about 1.6 times more C to SOC than did aboveground inputs (stover). Other studies supporting this conclusion include growth-chamber studies using  $^{14}\text{CO}_2$  tracers in oats (Gale and Cambardella 2000), field studies using  $^{14}\text{C}$  tracers in temperate deciduous forests (Norby et al. 2004), and field studies using root ingrowth

cores in tropical plantations Russell et al. 2004, 2007). Fine roots are difficult to study, and all methods for measuring fine-root growth have their limitations. Nevertheless, the body of evidence indicates that roots play a disproportionately important role in soil C sequestration in comparison to aboveground inputs. These results also suggest that the intimate association of roots within soil influences SOC stabilization in a fundamentally different manner than do aboveground components.

This study highlights the importance of incorporating both production and decomposition processes, as well as the location (above- or belowground) of detrital inputs into models of N-fertilization effects on soil C dynamics in agroecosystems. These results are highly relevant for evaluating the potential of N fertilization to mitigate the effects of removal of organic-matter “residue” from the system for bioenergy production. Our data suggest that the stimulation of OC decomposition by the addition of fertilizer N would likely counteract the positive effects of N fertilization on inputs of OC to the soil, at least for annual crops. Given the current quantity of N that is applied over such a large area, management strategies that can maintain high yields and also reduce N-fertilizer use would also have beneficial environmental consequences. Our study indicates that selection of crops for higher belowground NPP, in rotation with crops that fix N, could maximize both yields and soil C sequestration without excessive N-fertilizer additions.

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The authors declare that they have no competing financial interests.

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#### APPENDIX A

Tables showing aboveground and belowground net primary production of corn under four N-fertilization treatments in the Kanawha and Nashua experimental sites in Iowa, USA (*Ecological Applications* A019-043-A1).

#### APPENDIX B

Regression statistics for responses to N-fertilization treatments at two sites in Iowa, USA (*Ecological Archives* A019-043-A2).

#### APPENDIX C

The  $\delta^{13}\text{C}$  values of plant tissues and soil in three experimental cropping systems at Nashua, Iowa, USA (*Ecological Archives* A019-043-A3).