Uncertainty exists regarding the depth and extent to which agricultural practices affect soil properties, in particular soil organic C (SOC). In this study we examined the impact of 53 yr of continuous corn (Zea mays L.) receiving varying rates of inorganic N fertilizer with complete stover return on soil properties including SOC, total N, and bulk density (BD) to a depth of 1 m. In the treatment receiving virtually no applied N there was a significant reduction in soil N content at 0 to 30 cm over the study period, while the treatment receiving N in excess of recommended application levels was similar to the treatment receiving recommended rates of N fertilizer. Trends in SOC content were similar to those for total N, but a significant treatment effect was detectable throughout the entire 1-m soil profile sampled. Over the course of the study, all experimental fields appeared to have lost approximately 6 cm of topsoil through erosion, with the lowest N rate plots subsiding a further 2 cm due to compaction. Despite using disruptive management practices (moldboard plowing and application of anhydrous ammonia), declines in soil C and N content were not apparent for this soil type under conditions of low slope and the linear gains in productivity realized under the two higher N rate treatments. Thus N fertilizer was a benefit to this cropping system, rather than a detriment, and was sufficient to allow maintenance—but not building—of SOC.

**Abbreviations:** BD, bulk density; HI, harvest index; LOI, loss on ignition; NPP, net primary productivity; SOC, soil organic carbon; SOM, soil organic matter

The world’s soils contain roughly 1550 Pg of organic C—over twice the amount of C present in the atmosphere, and nearly three times that of aboveground biomass (Batjes, 1996; Lal, 2008). Globally, increased sequestration of C in agricultural soils holds potential to offset atmospheric CO₂ and contribute to mitigation of climate change (Lal, 2001, 2004; Smith et al., 2014). At local and regional scales, SOC positively impacts soil structure, water holding capacity, and fertility (Lal, 2008). While there is a clear need for management strategies that enhance SOC content, considerable uncertainty remains regarding soil C and N dynamics and the extent and depth to which they are influenced by agricultural practices including fertilization, rotation, and tillage.

Rates of N fertilization may affect SOC content through several mechanisms, including (i) influencing net primary productivity (NPP) and therefore C inputs to the soil system, (ii) altering plant above- and belowground partitioning of NPP and thus the vertical distribution of C inputs, (iii) causing changes to the C/N ratio of plant tissue which could affect its decomposition rate, and (iv) directly impacting microbial activity and rates of decomposition (Khan et al., 2007; Russell et al.,...
2009). Additions of N have been alternately observed to increase, decrease, or have no effect on SOC levels (e.g., Fog, 1988; Neff et al., 2002; Russell et al., 2005; Khan et al., 2007; Russell et al., 2009; Brown et al., 2014), depending on climate, soil type, and management (Alvarez, 2005). In particular, it has been argued that N fertilization beyond plant requirements is not only economically wasteful and environmentally deleterious, but that the practice may result in net loss of SOC due to promotion of plant tissue and soil organic matter (SOM) decomposition through enhanced microbial activity (Khan et al., 2007; Mulvaney et al., 2009). The relative importance of this phenomenon to overall SOC balances is debated, however, based on confounding factors of site history and ongoing SOC losses following cessation of historic applications of inorganic fertilizer (Powlson et al., 2010), and because N fertilization has elsewhere been found to slow the rate of SOC loss relative to no fertilization (Ladha et al., 2011). Such controversy highlights the uncertainty surrounding the impact of inorganic N fertilizer in particular on soil C and N dynamics, in contrast to the widely recognized positive impacts of organic fertilizers such as manure on SOC (e.g., Mulvaney et al., 2010; Powlson et al., 2010).

The impact of cropping system on soil C and N is likewise complex. While it is clear that soils of the upper Midwest experienced major losses of soil C on conversion from tallgrass prairie to agriculture (Huggins et al., 1998; Olson, 2013), responses of these soils under continued cultivation are varied. David et al. (2009) confirmed large reductions in both SOC and total N on initial conversion, but observed no significant change in soil C and N quantities under contemporary systems of the 1950s and beyond. Additional studies have also reported general stability of SOC levels, as well as instances of loss and of gain associated with a variety of rotations and management practices. Russell et al. (2005), for instance, noted that depending on soil type, rotations including soybean [Glycine max (L.) Merr.] experienced either nonsignificant decreases or minor increases in SOC stocks, while rotations including alfalfa (Medicago sativa L.) experienced significant increases in SOC. In contrast, Sanford et al. (2012) observed losses in SOC content across a variety of cropping systems over 20 yr, including from alfalfa-based rotations. Prior to that study's inception, however, the site had received regular manure application, which may explain the universal losses. In continuous corn rotations, positive, negative, and neutral rates of change in SOC stocks have all been reported (Huggins et al., 1998; Allmaras et al., 2004; Khan et al., 2007; David et al., 2009; Sanford et al., 2012; Brown et al., 2014).

Soil organic C and N contents are also influenced by depth and tillage. While a preponderance of studies exploring the impacts of management on SOC and N focus on the plow layer (the surface 30 cm) where SOM is typically most highly concentrated, a significant amount of SOC and N is also present in deeper soil layers where less is known about the impact of land use and management practices on C and N storage (Batjes, 1996; Lorenz and Lal, 2005; Olson et al., 2014). Notably, Gál et al. (2007) found that a no-till treatment resulted in significantly higher SOC and N contents than did moldboard plow in the surface 15 cm, but that the effect was reversed at a depth of 30 to 50 cm. Little significant difference was seen at depths below 50 cm, and while a 30-cm sampling depth showed 23 Mg ha⁻¹ greater SOC content under no-till compared with moldboard plow, a sampling depth of 1 m reduced the difference to just 10 Mg ha⁻¹. Other studies suggest that cultivation-induced changes to subsoil C and N are sometimes but not always present, and that changes to SOC distribution within the soil profile may occur even while overall content remains constant (Collins et al., 2000; VandenBygaart et al., 2003; David et al., 2009; Luo et al., 2010; Olson et al., 2014). With maximum crop rooting depths averaging approximately 2 m globally (Canadell et al., 1996), subsoil root C deposition (rhizodeposition) and nutrient and water acquisition are important aspects of overall C sequestration and system performance, and improved understanding of the response to management of both surface and subsoil C and N is needed.

With nearly 37 million hectares of corn harvested in North America in 2012 and over 178 million hectares harvested globally, corn accounts for approximately 18 and 13% of North American and global arable land use, respectively, and is among the world's most widely planted crops (FAOSTAT, 2015). This ubiquity makes corn an excellent standard with which to study cropping system and soil responses to management practices. Furthermore, increased interest in intensive corn production due to its value as a biofuel feedstock has raised questions about the sustainability of yield and maintenance of soil quality in such systems—particularly continuous corn receiving inorganic N fertilizer—and the role of stover in maintaining SOC stocks (Graham et al., 2007; Wilhelm et al., 2007; Landis et al., 2008; Williams et al., 2009).

Here, we have examined soil properties to a depth of 1 m as impacted by a long-term experiment involving 53 yr of continuous corn production with variable inorganic N rates and complete stover return. The system has been under aggressive management, both physically and chemically, with tillage by moldboard plow and the majority of N applied in the form of anhydrous ammonia. Previous studies based on this trial have found that while treatments receiving N approximately at recommended (Rec. N) and higher than recommended (High N) rates have generally kept pace with national trends of increase in corn yield, the treatment receiving a low N rate (Low N) has experienced virtually no yield increase—but no decrease either—since the trial's inception in 1958 (Bundy et al., 2011). Intriguingly, Motavalli et al. (1992) observed that after 25 yr of N rate treatments within this trial, cessation of fertilization in subplots resulted in a rapid decrease in soil nitrate in the Rec. N and High N treatments to contents comparable with the Low N treatment, but that grain yields in the Rec. N and High N treatments continued to surpass those of the Low N treatment for up to 7 yr. These findings indicate that higher N rates resulted not only in temporary increases to soil nitrate, but also substantial retention or buildup of mineralizable N, suggesting a positive impact of...
N rate on SOM and thus SOC. Specific objectives of this study, therefore, were to examine the effect of long-term fertilizer N rate, as well as historic application of lime, on the quantity and quality of soil C, N, and other soil properties, and to determine to what depth treatment effects are detectable. We further sought to determine whether N application in excess of plant demand has negatively affected SOC content, and to explore the impact of the long-term treatments on erosion and compaction.

MATERIALS AND METHODS

Site Characteristics and Experimental Design

The study was conducted at the University of Wisconsin–Madison Arlington Agricultural Research Station located in south-central Wisconsin, USA (43°18’ N lat; 89°21’ W long). Soil at the site is a Plano silt loam (Typic Argiudoll) developed under prairie vegetation on loess over glacial till with a 0 to 1% slope. Average annual temperature and precipitation are 6.9°C and 869 mm, respectively (National Centers for Environmental Information, 2015). Historical treatments are described in detail in Bundy et al. (2011). In brief, prior to the start of the long-term trial, the site had been farmed with “a minimum of good soil management” for approximately 25 yr (Andrew et al., 1963). In 1958, the experiment was established consisting of continuous corn receiving variable rates of inorganic N fertilizer, with treatments laid out in a randomized complete block design with four replications. For the duration of the study, grain was harvested and all residue was returned to the soil by moldboard plow. Seeding rate, hybrid selection, and other management practices have been in keeping with prevailing best practices (Bundy et al., 2011). Primary long-term treatments consisted of three fertilizer rates: Low N, which received only annual starter fertilizer at an average rate of 10 kg N ha⁻¹; Rec. N, which received a N rate in the range of agronomic recommendations, and High N, which received N well in excess of recommended rates. Precise rates and composition of fertilizer have varied over the course of the study to keep pace with typical best practices; from 1958 to 1992 the Rec. N treatment received between 56 and 168 kg N ha⁻¹ annually and the High N treatment received between 112 and 252 kg N ha⁻¹ annually, with consistent rates of 140 and 280 kg N ha⁻¹ yr⁻¹ for Rec. N and High N treatments, respectively, thereafter (Bundy et al., 2011). Although recommended N rates for corn in the state of Wisconsin have increased over the past several decades and can now exceed 200 kg N ha⁻¹ yr⁻¹ under optimal soil and market conditions (Laboski and Peters, 2012), the lack of significant difference in grain yield between Rec. N and High N treatments (Bundy et al., 2011) suggests that the Rec. N treatment has received N at a rate that is near-optimum for this location and soil. From 1984 to 1992 each of the twelve 12 × 60 m plots was divided into four subplots with variable N rates to study residual effects of long-term treatments (Motavalli et al., 1992), resulting in forty-eight 12 × 15 m subplots. In 1985 and again in 1988, one half of each long-term treatment was limed to achieve a pH of 6.5 to 7.0, resulting in a total of ninety-six 6 × 15 m sub-subplots. The present study considers only those subplots whose N rates were consistent with the three primary long-term treatments, with samples collected from Low N, Rec. N, and High N sub-subplots having received lime as well as unlimed Rec. N sub-subplots. Rec. N refers to the limed treatment except where otherwise noted.

Soil Sampling and Analysis

Soil samples were collected on 5 May 2011 using a hydraulic soil sampler fitted with a 4.3-cm diam. probe. Three cores were collected per sub-subplot and sectioned into depth increments of 0 to 5, 5 to 10, 10 to 15, 15 to 30, 30 to 60, and 60 to 100 cm. Depth of the A horizon was recorded during core processing, and BD was calculated for each depth increment of each core. After sectioning, subsamples were dried at 105°C for determination of moisture content (Topp and Ferré, 2002), and the remainder was dried in a 35°C forced-air oven and ground to pass a 2-mm sieve. For determination of pH and SOM, P, and K content, composite samples were created from each of three cores for each sub-subplot and depth increment, and analyzed at the University of Wisconsin Soil Testing Laboratories. Soil organic matter content was determined by loss on ignition (LOI) at 360°C for 2 h (Combs and Nathan, 2015), and plant-available P and K were determined by Bray-1 (Bray and Kurtz, 1945; Munter, 1988). For determination of total C and N content, approximately 2 g of sample from each depth of each core was further ground to a fine powder using a ball mill, and 8 to 10 mg was analyzed by dry combustion in an automatic elemental analyzer (Flash EA 1112 CN, Thermo Finnigan). Total C and N samples were run in triplicate and average values used for further analysis. Inorganic C in these soils has been previously shown to be negligible (Paul et al., 2001), thus total C is equivalent to total organic C. Archived 0- to 15-cm samples from 1984 were also re-analyzed for total C and N by dry combustion as described above, using one 8- to 10-mg subsample from each of three samples per sub-subplot.

The site is surrounded by an unplowed and unsowed grassland that is frequently moved and experiences some truck and tractor traffic. Depth of this field edge A horizon was measured with a hand probe in October 2013 (n = 17). In July 2015, three samples were collected from each side of the field edge (n = 12) with a hydraulic soil sampler fitted with a 4.3-cm diam. probe. The entire A horizon was retained from each core and dried at 150°C to calculate A horizon mass.

Elevation Mapping

Field elevation was measured by differential leveling in November 2013, prior to tillage, using a level, tripod, and measuring rod. Elevation was measured in all sub-subplots within the experimental field, grouped by long-term N rate (regardless of short-term N rate or lime treatment). Four measurements per sub-subplot were made along a diagonal transect excluding the outermost meter of each, and sub-subplot averages were used for further analysis. Measurements were also recorded at the same density for the grassy field edge and for each of 12 permanent markers surrounding the field. To adjust for the natural roll of
the land (which varies by up to 1.07 m across the field), data was standardized along East–West and North–South axes, as well as by quadrant. NAVD88 values were calculated based on the nearby National Geodetic Service benchmark 2U72.

Calculations and Statistical Analysis

Estimates of C and N return and removal with corn biomass, grain, and fertilizer were conducted. The US corn harvest index (HI) has not changed significantly over the course of the study (increases in corn yield potential are associated with increases in overall biomass production rather than a shift in the ratio of grain to stover; Hay, 1995; Sinclair, 1998), therefore we applied a HI of 0.5 to historical as well as contemporary yields to estimate the amount of aboveground biomass returned to the system. We applied a ratio of belowground biomass input (root biomass + rhizodeposition) to aboveground biomass production (grain + stover biomass) of 0.6 (Amos and Walters, 2006; Johnson et al., 2006) to estimate total biomass entering the system annually, and a biomass C content of 0.45 (Vanotti et al., 1997) to estimate C inputs to the system. That is, total C input = 0.45 × [stover biomass + (aboveground biomass × 0.6)]. Nitrogen removed as grain was estimated based on a grain N content of 11.5, 13, and 13.5 g N kg⁻¹ for Low N, Rec. N, and High N treatments, respectively. These values correspond approximately to corn grain N contents when fertilizer is applied 150 kg ha⁻¹ below, at, and 150 kg ha⁻¹ above the economic optimum N rate, respectively, as reported by Cerrato and Blackmer (1990). Rates of return were estimated by linear regression based on annual grain yield as reported in Bundy et al. (2011) and Supplemental Table S1. Missing yield data for 1963 to 1967 was inferred based on linear regression equations for each long-term N treatment. Regression equations were: y = 0.0149x – 25.55 for Low N, y = 0.156x – 300.36 for Rec. N, and y = 0.153x – 293.56 for High N, where y = Mg ha⁻¹ grain at a moisture content of 155 g kg⁻¹ and x = year.

A SOC to SOM conversion factor was calculated for each sub-subplot at each depth by dividing the SOM content by the SOC content. The BD and SOC and N content of each depth increment sampled were used to calculate C and N stocks by both linear depth (0–100 cm) and equivalent soil mass (mass depth, 135 g cm⁻²) for each treatment. Mass depth was calculated by either decreasing or extending, as appropriate, the quantity of 60- to 100-cm soil included in the calculation to achieve a mass per area of 135 g cm⁻². Mass of the A horizon was calculated based on BD of each depth increment in which the A horizon was present. Bulk density values were weighted by the length of the increment (5, 15, or 30 cm). For the depth increment in which the A horizon ended (15–30 or 30–60 cm), the BD of that increment was weighted based on the length to which the A horizon extended into its range.

The historical comparisons presented here are limited to 1984 samples, which were available for re-analysis of C and N content by dry combustion. We chose not to consider values reported for 1958 (samples not available for re-analysis), because those original values were determined via the Walkley–Black procedure and a SOC to SOM conversion factor of 1.724 (Vanotti et al., 1997; M.B. Vanotti, personal communication, 2014). As this technique is subject to variation and error associated with incomplete digestion and the necessary use of a correction factor to estimate true SOC content, precision of independent historical values are less reliable than are conclusions drawn from treatment comparisons. To further minimize error, we limited our historical comparison with C and N content rather than deriving values for C and N stocks based on assumptions about historical BD. This approach is in keeping with recommendations of Lee et al. (2009).

Statistical analysis was conducted with SAS v.9.4 (SAS Institute, 2015). For statistical purposes the whole plot treatments are either the four N and lime rate treatments or the three N rate treatments, depending on the measured variable. All soil data collected with depth (total N, SOC, C/N, SOM, SOC to SOM conversion factor, BD, pH, P, and K) was analyzed with ANOVA using Proc MIXED with the four N and lime treatments and depth as fixed effects, block as a random effect, and depth as a repeated measure (REPEATED, SUBJECT = block by N rate). Significant differences among the four N and lime treatments were determined with LSMEANS by depth using SLICE. Linear and quadratic contrasts were used to determine the nature of the response to soil variables across N rates or lime addition (linear only) by depth with Proc MIXED (block as a random effect) using ESTIMATE function. Proc MIXED was also used to determine the effect of long-term N rate on linear and mass depth of total N and SOC, elevation, A horizon depth, and A horizon mass with treatment differences determined with LSMEANS. Change in N and C content and C/N of surface soils between 1984 and 2011 were determined using Proc MIXED with three N rate treatments and year as fixed effects, block and block × N rate treatment as random effects and year as a repeated measure (REPEATED, SUBJECT = block × N rate) and LSMEANS. All significance is reported at the α = 0.1 significance level unless otherwise noted. The assumption of normally distributed errors was met for all variables.

RESULTS

Effects of Nitrogen Rate on Soil Properties by Depth

Of the 54 combinations of soil property by depth (9 soil properties, 6 depths), N rate was found to have a significant effect in 27 cases using a linear contrast and in 14 cases using a quadratic contrast (Table 1). There were only five instances, distributed across different soil properties, in which the quadratic contrast was either the only source of significance or yielded a lower P-value than the linear contrast, indicating that the effects of N rate in this study generally most closely follow a linear relationship.

The Low N treatment resulted in significantly lower total N contents than the Rec. N treatment at 0 to 15 cm, and than the High N treatment at 0 to 30 cm, while Rec. N and High N treatments did not differ significantly in soil N content at any depth (Fig. 1a). Contents of SOC were also significantly lower
in the Low N than the Rec. N and High N treatments for every depth (0–100 cm), with the exception of 30 to 60 cm where a significant difference was detected only between the Low N and High N treatments (Fig. 1b). As with soil N content, the Rec. N and High N treatments did not differ significantly in SOC content at any depth. Similar relationships between N rate treatments were also observed for SOM content, also persisting to a depth of 1 m, with significance detected from 30 to 100 cm (Fig. 1d). The soil C/N ratio was generally lower in the Low N than Rec. N and High N treatments, and slightly though not significantly higher in the High N than the Rec. N treatment (Fig. 1c). Collectively, these results suggest that, although total N, SOC, and SOM content all decrease with decreasing fertilizer rate, the effect is proportionally greater on C than on N. This may be due in part to a change in SOM composition between treatments, which is supported by the increased SOC to SOM conversion factors of the Low N treatment compared with the Rec. N and High N treatments (Fig. 1e). The effect of N rate treatment on C/N ratio and SOC to SOM conversion factor remained consistent throughout the soil profile. However, for all treatments these factors decreased and increased, respectively, with depth below 30 cm (Fig. 1c and 1e). Our findings yield a SOC to SOM conversion factor in the range of 1.8 to 2.5 with a median of 2.0 (corresponding to a 50% C SOM composition) at 0 to 30 cm, rising to a range of 5.1 to 5.9 (median 5.3, corresponding to 19% C SOM composition) at 60 to 100 cm.

Bulk density was significantly higher in the Low N than the Rec. N and High N treatments at 5 to 30 cm. At 0 to 5 cm BD was significantly lower in Rec. N treatment than the Low and High N treatments, but no significant difference in BD was observed between Rec. N and High N treatments at any other depth (Fig. 1f).

Long-term N rate had no significant effect on pH, P content, or K content at most depths (Table 1, Supplemental Figure S1), with few discernable trends. One notable exception was a higher P content in the Low N treatment from 0 to 15 cm (significantly higher than the High N treatment from 0 to 10 cm), likely due to greater P uptake where fertilizer N was applied as a result of greater yield. Another was the consistent but nonsignificant trend of higher pH in Low N than in Rec. N and High N treatments from 0 to 30 cm (P-values ranged from 0.15 to 0.23 for the linear effect).

Effects of Lime Treatment on Soil Properties by Depth

Out of the 54 combinations of soil property by depth examined here, there were only 3 instances in which lime treatment was found to have a significant effect; the C/N ratio differed significantly at the 15- to 30-cm depth (P = 0.067, +Lime = 10.46, −Lime = 9.22), BD differed significantly at the 0- to 5-cm depth (P = 0.093, +Lime = 0.95 g cm⁻³, −Lime = 1.04 g cm⁻³), and P content also differed significantly at the 0- to 5-cm depth (P = 0.095, +Lime = 34.50 mg kg⁻¹ soil, −Lime = 23.83 mg kg⁻¹ soil). Of note is the lack of significant effect of the lime treatment on soil pH at any depth, although from 0 to 60 cm there was a tendency for pH to be slightly lower in the −Lime than the +Lime treatment. It should be kept in mind, however, that lime had not been applied since 1988.

Carbon and Nitrogen Stocks

Bulk density, SOC, and total N content by depth increment were used to calculate SOC and N stocks at 0 to 100 cm (Table 2). These calculations showed significantly higher overall C stocks in Rec. N and High N treatments than in the Low N treatment, with no significant difference between Rec. N and High N treatments or between limed and unlimed treatments. Differences in N
Soil properties of three long-term N rate treatments with depth. Data are displayed at the median of the sampling range. Significant differences are indicated at right (L, Low N; R, Rec. N; H, High N; NS, no significant difference between treatments), determined from differences in least squares means ($\alpha = 0.1$).

Fig. 1. Soil properties of three long-term N rate treatments with depth. Data are displayed at the median of the sampling range. Significant differences are indicated at right (L, Low N; R, Rec. N; H, High N; NS, no significant difference between treatments), determined from differences in least squares means ($\alpha = 0.1$).

stocks were not significant, although N mass was slightly lower in the Low N treatment compared with the Rec. N and High N treatments, which were virtually indistinguishable.

Because of the effect of long-term N rate on BD, with Low N exhibiting greater compaction (Fig. 1f), use of the linear depth (0–100 cm) approach may overestimate C and N stocks of the Low N treatment relative to others due to inclusion of greater soil mass in the volume being described. We therefore also calculated C and N stocks for a soil mass depth of 135 g cm$^{-2}$, which was in the mid-range of calculated 0 to 100 cm soil masses across treatments. This analysis yielded a similar relationship among treatments as the linear depth approach (Table 2). However, while standardization by linear depth resulted in C content values 16 and 23% higher for Rec. N and High N, respectively, compared with the Low N treatment, standardization by mass depth resulted in ratios of 18 and 26% for the same comparison. Thus, failure to account for varying degrees of compaction between treatments using the linear depth approach results in 2 and 3% underestimation of the difference between Rec. N and Low N treatments and High and Low N treatments, respectively.

Changes in the A Horizon

To further explore the effect of long-term N rate on soil compaction, we performed elevation mapping of the experimental field. All 96 sub-subplots within the trial were measured and grouped by long-term N rate ($n = 32$). In keeping with BD
measurements, Rec. N and High N treatments were virtually indistinguishable, while elevation in the Low N treatment was approximately 2 cm lower (Fig. 2a). These findings are further supported by depth of the A horizon (Fig. 2b), which reveals the Low N treatment to have an A horizon approximately 2.5 cm shallower than other treatments. It is important to note that the soil mass of the A horizon, as calculated based on depth and bulk density, did not differ significantly between treatments ($P = 0.972$, Fig. 2c)—suggesting that the reduction in elevation and A horizon depth observed in the Low N treatment is due primarily to compaction rather than soil loss through erosion or other mechanisms. This minor offset between depths described in the Low N treatment and those of the Rec. N and High N treatments (Fig. 3) is not expected to greatly impact treatment comparisons by depth, because of the lack of a perceptible depth effect at 0 to 15 cm (Fig. 1) and the dilution effect of larger sampling increments below 15 cm.

Notably, over the course of the trial the entire experimental field has visibly subsided compared with the uncultivated field edge—a phenomenon also revealed by both elevation mapping and A horizon depth, where the field edge is approximately 6 cm higher with a 6 cm deeper A horizon than Rec. N and High N treatments (Fig. 2a, 2b, and 3). Although the field edge has been under no particular management regime and field edge soil samples were collected several years after those from experimental plots, it is nonetheless interesting to note that the BD of the field edge A horizon (1.1 g cm$^{-3}$) is in the same range as in experimental plots (Fig. 1f), and thus compaction is not likely the primary cause of the height difference between the experimental field and field edge. The mass of the field edge A horizon is, however, substantially greater than in experimental treatments (Fig. 2c), suggesting that loss of surface soil has occurred across the entire experimental field over the course of the study, that the field edge has experienced more building of topsoil than the experimental field, or both.

**Change in Biomass Inputs and Soil Carbon and Nitrogen over Time**

While grain yield from Rec. N and High N treatments has more than doubled over the course of the study (increasing from roughly 4 to over 14 Mg ha$^{-1}$, at a rate of approximately 150 kg ha$^{-1}$ yr$^{-1}$), yield of the Low N treatment has remained relatively constant in the range of 2 to 6 Mg ha$^{-1}$ (Supplemental Table S1; Bundy et al., 2011 Tables 3 and 4). Using standard conversion factors for HI, above- vs. belowground biomass partitioning, biomass C content, and grain N content, C and N removed from and returned or applied to the system was estimated (Table 3). While there was initially little difference in bio-

| Treatment | $0$–$100$ cm | $135$ g cm$^{-2}$ | $0$–$100$ cm | $135$ g cm$^{-2}$ |
|-----------|--------------|------------------|--------------|------------------|
| Low N     | 87.0a†       | 85.8a            | 11.4         | 11.2             |
| Rec. N    | 101b         | 101b             | 11.9         | 12.0             |
| High N    | 107b         | 108b             | 11.9         | 12.0             |

† Values within each column followed by the same letter are not significantly different ($\alpha = 0.1$). Connecting letters are shown only where significant differences exist within a column.
mass return between treatments, over the course of the study returns from the Rec. N and High N treatments have risen at an estimated rate of roughly 0.13 Mg C ha−1 yr−1 so that after 53 yr annual C inputs to the Low N and High N treatments are predicted to be, respectively, 34 and 103% that of the Rec. N treatment. Between 1958 and 2010, the Low N and High N treatments are estimated to have, respectively, net N balances -76 and 367% that of the Rec. N treatment, and net C balances 46 and 105% that of the Rec. N treatment (Table 3).

To investigate change in soil C and N content over time, we compared samples from 2011 and 1984. Although C content had been measured in 1984 (Vanotti et al., 1997), the modified Mebius procedure used at that time can be subject to greater variability than the current dry combustion method, and use of 1984 reported results would not have allowed statistical comparison with 2011 samples. We therefore re-analyzed archived 0- to 15-cm samples from 1984 for C and N content by dry combustion. While Rec. N and High N treatments saw no significant change in either N or C between 1984 and 2011, contents of both elements decreased markedly in the Low N treatment (Fig. 4a and 4b), indicating that SOC and N have been lost from the Low N system over time. Interestingly, the change in C has been proportionately greater than the change in N, a relationship that is reflected by the decrease in C/N ratio over time (Fig. 4c).

**DISCUSSION**

**Depth of Detectable Treatment Effects**

Fifty-three years of minimal N input (10 kg ha−1 yr−1) in a continuous corn cropping system has increased BD and lowered SOC, total N, and SOM contents compared with fertilized treatments, with differences between treatments discernable to depths as great as 1 m in some soil properties. Soil organic C differences were observed at the deepest depth sampled, where at 60 to 100 cm the SOC content in the Low N treatment was approximately 18% less than in the Rec. N treatment (Fig. 1b). A divergence of such magnitude over only 53 yr is noteworthy, considering the 282-yr mean 13C residence time reported at 50 to 100 cm for this location by Collins et al. (1999), which would translate to roughly 20% SOC turnover during this study’s timeframe given exponential decay \[ A_t = A_0 e^{-kt} \]

![Fig. 3. Adjusted alignment of sampling depths relative to A horizon for each long-term N rate treatment and field edge. Depths are based on measured A horizon depths and are drawn to scale.](image)

Table 3. Estimated C and N balances for long-term N rate treatments 1958 to 2010.

| Treatment | Input† | Removed‡ | Net   |
|-----------|--------|----------|-------|
| Low N     | 0.5    | 2.1      | -1.6  |
| Rec. N    | 7.3    | 5.2      | 2.1   |
| High N    | 13.3   | 5.6      | 7.7   |
| Low N     | 181    | 82       | 99    |
| Rec. N    | 395    | 180      | 215   |
| High N    | 414    | 188      | 226   |

† Based on fertilizer applications for N, and on stover, belowground biomass, and rhizodeposition for C, with estimates as follows: harvest index = 0.5, belowground biomass input to aboveground biomass production = 0.6, biomass C content = 0.45 (Hay, 1995; Vanotti et al., 1997; Sinclair, 1998; Amos and Walters, 2006; Johnson et al. 2006).
‡ Based on grain N and C content, with estimates as follows: grain C content = 0.45, grain N content = 0.0115, 0.013, and 0.0135 for Low N, Rec. N, and High N, respectively (Cerrato and Blackmer, 1990).

Table 4. Contribution of surface (0–30 cm) and subsoil (30–100 cm) to differences in SOC stocks between long-term N rate treatments, based on measured C content, bulk density, and linear depth.

| Treatment | 0–30 cm | 30–100 cm | Proportion at 30–100 cm relative to total 0–100 cm |
|-----------|---------|-----------|-----------------------------------------------|
| Low N     | 50.8    | 36.2      | 42                                            |
| Rec. N    | 58.6    | 42.3      | 42                                            |
| High N    | 61.1    | 46.3      | 43                                            |
| Low N- Rec. N | -7.8 | -6.1      | 44                                            |
| High N- Rec. N | 2.5  | 4.0       | 61                                            |
at 30 to 100 cm (Table 4), a proportion consistent with those reported by Gray et al. (2015) for wet climate zones in eastern Australia. Omission of 30- to 100-cm subsoil data in the present study would have resulted in failing to account for roughly half of the differences in C stocks between treatments.

It is noteworthy that significant impacts of long-term N rate at the 30- to 100-cm depth were detected not only on SOC content but also on SOM content and the SOC to SOM conversion factor (Fig. 1d and 1e). Thus the phenomenon of relatively carbon-poor SOM resulting from the Low N treatment compared with other treatments is not confined to the tillage zone but continues into the subsoil. Results presented here additionally suggest a considerable decrease in the C/N ratio and SOM C content with depth (Fig. 1c and 1e). Some of these differences could be artifacts of the LOI method and increasing clay content, and/or mineral N sorbed to clay particles at depth (Pribyl, 2010; Rumpel and Kögel-Knabner, 2010). The decrease in C/N ratio with depth observed here is, however, consistent with the findings of others, and likely indicative of more highly processed SOM (Russell et al., 2005; Rumpel and Kögel-Knabner, 2010; Veenstra and Burras, 2015). These observations also support a more nuanced view of the conversion of SOC to SOM than the conventionally employed factor of 1.724, which is based on the assumption that SOM contains 58% C. As reviewed by Pribyl (2010), this factor can in fact range widely and may be influenced by depth as well as by the method used to determine SOM content. Thus the relationship between SOC and SOM should not necessarily be assumed to be constant—a consideration that is of particular relevance for long-term studies where past analytical techniques underpinning historical data have relied on this conversion factor.

**Effect Over- and Under-Application of Nitrogen on Soil Carbon and Nitrogen**

The lack of significant difference at any depth between Rec. N and High N treatments in SOC and N content is noteworthy. This suggests that no acceleration of net mineralization due to continual over-application of inorganic N fertilization has taken place within the High N treatment in this trial. It is technically possible, however, that minor increases in mineralization with N over-application could be masked by the slightly higher biomass inputs associated with the marginally though not significantly higher productivity of the High N treatment. The slight though nonsignificant increase in C/N ratio for the High N compared with the Rec. N treatment (Fig. 1c) is also notable, suggesting that any decrease in biomass C/N as a result of increased rates of fertilization, as reported by Russell et al. (2009), were overshadowed by processes within the soil—possibly including accelerated rates of decay. Therefore while we cannot rule out the existence of a slight increase in N mineralization associated with N over-application, we find no direct evidence of a negative relationship between inorganic N application and soil C and N such as that suggested by Khan et al. (2007) and Mulvaney et al. (2009). Rather, our results describe a positive-to-neutral association between N fertilization rate and soil C and N content, in keeping with the conclusions of Powlson et al. (2010) and Ladha et al. (2011).

Our results are also in keeping with those of Brown et al. (2014), who found no effect of N over-application on SOC in a similar system of continuous corn with stover return. Interestingly, our results differ from Brown et al. (2014) in the effect of low N rates on SOC. Whereas we observed a significant difference in SOC content between the Low N treatment and Rec. and High N treatments (Fig. 1), Brown et al. (2014) report no effect of low N rates on SOC, despite similar levels of stover biomass return (averaging 3.6 Mg dry matter ha\(^{-1}\) yr\(^{-1}\) in
Brown et al. (2014) and 3.4 Mg dry matter ha\(^{-1}\) yr\(^{-1}\) in the present study). This contrast between studies adds to the general understanding that the response of SOC to rates of fertilization and biomass return is not always constant, but depends on numerous other system variables such as climate, soil type, tillage, and timeframe of observation (Alvarez, 2005; Russell et al., 2009).

Defining Depth

Through measuring surface elevation, A horizon depth, and BD (Fig. 1 and 2), we were able to determine that significant compaction has occurred in the Low N treatment relative to the Rec. N and High N treatments, to the extent that a sampling depth of 1 m did not represent the same mass of soil or absolute vertical location across treatments (Fig. 3). We accounted for this discrepancy by performing adjustments based on BD. Calculations of C stocks in terms of linear depth as well as mass depth (Table 2) reveal compaction to be a potential source of error if left unaccounted for in the reporting of C with depth, and support the use of an equivalent soil mass approach rather than a fixed depth approach in estimating soil C stocks (Lee et al., 2009). The reduction in the surface elevation of the experimental field relative to the surrounding grassland was also noteworthy (Fig. 2 and 3), with A horizon mass indicating a discrepancy of over 250 Mg ha\(^{-1}\) caused either by erosion, diminished soil building activity, or a combination of those two factors.

Although nonsignificant differences in BD were discernable from 30 to 100 cm in this study, all significant differences occurred within the surface 30 cm, roughly corresponding to the depth of the A horizon as well as the plow layer. Thus, depending on study and site parameters, location of the A to B horizon transition presents a useful diagnostic tool, and standardization of sampling depth based on location of the transition could provide a useful relative depth approximation when BD data is not available. It has also been noted, however, that the soil-mixing action of deep tillage regimes may result not only in diffusion of C and N within surface horizons but also in an increase in the depth to which mollic colors are evident in the soil profile (Veenstra and Burras, 2015). We find no evidence of tillage-induced deepening of the A horizon in the present study, however, given the consistent relationship between the experimental field and the field edge as revealed by both elevation mapping and measurement of A horizon depth (Fig. 2a and 2b). Nevertheless, it may be that the utility of A horizon depth as an indicator of vertical position would best be confined to comparisons within a common tillage regime.

Effect of Nitrogen Fertilizer on Soil Carbon and Nitrogen Stability Over Time

The Low N treatment, which has experienced a net negative N balance over the study period, has also experienced a decline in N content in the 0- to 15-cm depth over the 28-yr period from 1984 to 2011 (Table 3, Fig. 4a). Conversely, the High N treatment, which has a positive N balance—having received over 25 times more N fertilizer than the Low N treatment over the study period, has experienced no change in N content since 1984.

Finally, while the positive N balance of the Rec. N treatment is less than that of the High N treatment, no change over time or difference between the two treatments was discernible in N content (Table 3, Fig. 4a). Thus while severely restricted N inputs have resulted in continuing losses of soil N, neither adequate nor excessive N inputs have achieved appreciable increases in total soil N content.

A similar trend over time to that of total N content is evident in SOC content, with the Low N treatment experiencing a significant decrease but with no significant change observed in Rec. N and High N treatments between 1984 and 2011 (Fig. 4b). The loss of SOC in the Low N treatment contradicts its apparently positive net C balance (Table 3), and it should be noted that soil respiration is not included in C balance and likely accounts for the discrepancy. The relatively constant, low rate of C input to the Low N system has not been sufficient to maintain SOC levels, while the increasingly higher rates of C input to the Rec. N and High N treatments (due to increasing productivity over time) has not thus far resulted in any perceptible increase in SOC. If anything, SOC content appears to have declined, though not significantly, since 1984 in the Rec. N and High N treatments (Fig. 4b). Without additional data points between 1984 and 2011, however, it is impossible to determine whether these treatments are in a steady state, or whether limited observations in fact conceal a curvilinear trend driven by constantly increasing rates of C input. An initial trend of decline followed by a reversal is conceivable given estimated C input rates, but more detailed historical data would be needed to draw any conclusions beyond speculation. Of additional note is the significant reduction in C/N ratio at 0 to 15 cm in both Low N and Rec. N treatments between 1984 and 2011 (Fig. 4c). This could indicate a greater proportion of highly processed, older SOM (Baisden et al., 2002; Rumpel and Kögel-Knabner, 2010), possibly pointing to subtle changes in soil properties within the Rec. N treatment that are not yet evident in measures of total N and SOC.

It is further interesting to note that the apparent stability of SOC levels in the Rec. N and High N treatments stand in distinct contrast to significant losses of SOC observed over 20 yr of continuous corn receiving N fertilizer at recommended rates in a nearby long-term experiment (Sanford et al., 2012). In that experiment, also at the Arlington Agricultural Research Station and on the same soil type, losses are attributed to a history of management practiced prior to 1958 (Andrew et al., 1963), we might have observed different trends over time.
Perceptible differences in soil properties at depths as great as 1 m reported here underscore the importance of including subsoil in examinations of the long-term effects of land management on SOC dynamics and C sequestration. In this long-term trial, the robust productivity of Rec. N and High N treatments and the relatively high indicators of soil quality in these treatments compared with the Low N treatment create an appearance of stability. Yet close examination of soil C levels compared with inputs over time suggests that even these highly productive treatments may be unable to build SOC, and calls their stability into question—if productivity were to plateau, would SOC content begin to decline? An uncultivated site near the study field was reported to have a soil C content of 22.6 g kg⁻¹ (Vanotti et al., 1997), compared with the 18.6 g kg⁻¹ in the top 20 cm of the High N treatment in 2011. While contemporary data from surrounding ‘pristine’ locations cannot fully replace knowledge of pre-European conditions, this comparison suggests that even in the High N treatment, current SOC levels are well below the soil’s potential for C storage, and will not be rebuilt under the current system of continuous corn receiving inorganic N fertilization.

CONCLUSIONS

This study applies both physically (moldboard plow) and chemically (anhydrous ammonia) disruptive methods in a continuous corn cropping system, yet results show that even with this management system, declines in soil C and N content are not apparent for this soil type under the conditions of low slope and linear gains in yield. Thus N fertilizer was a benefit to this cropping system, through positive feedback, rather than a detriment. The long-term effect of N application can be detected throughout the soil profile, with minor but measureable difference detected to 1 m. Changes in the C/N ratio also occurred among treatments and over time, suggesting changes to SOM quality and biochemical properties. Overall, this production system has led to a reduction in soil elevation and mass, with further losses occurring without additions of N fertilizer. Results of this study highlight an important contrast between improvement of yield or maintenance of soil quality, and demonstrates that the former may be achieved without the latter, at least in the half-century timeframe of this long-term trial. Ultimately, the sustainability of cropping systems must be defined on much longer time horizons, and relatively short-term gains in annual productivity must be balanced with longer-term stability and resilience, to which features such as those included in the concept of soil health are key.

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SUPPLEMENTAL MATERIALS

Supplemental Fig. S1 shows soil pH, P content, and K content of three long-term N rates with depth. Supplemental Table T1 shows effect of long-term N rate and lime on corn grain yield 2008 to 2010.

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