Nitrogen and carbon dynamics in prairie vegetation strips across topographical gradients in mixed Central Iowa agroecosystems

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Abstract
Reductions of nitrogen (N) export from agricultural lands because of changes in specific N stocks and fluxes by incorporation of small amounts of prairie vegetation strips (PVS) are poorly understood. The primary objective of this study was to evaluate the effect of the presence and topographical position of PVS on soil and plant carbon (C) and N stocks relative to annual crop and native prairie vegetation. The study was implemented within three small adjacent watersheds, treated with one of the following cover types: (1) 100% row-crop agriculture (CROP); (2) 20% prairie vegetation (PVS) distributed along the contour across three topographical positions: upslope, sideslope and footslope position; and (3) 100% 17-year old reconstructed native prairie (RNP) as the control condition. Total soil organic C (SOC), total soil N (TN), inorganic N availability as indexed by ion exchange resins, N stocks in plant biomass and litter, and the ratio of C3:C4 plant species were measured during the 2010 growing season. Results showed that over five years of treatment, PVS footslope improved soil quality by increasing TN by almost 100% and SOC by 37%; while CROP footslope TN decreased by 31% and SOC decreased by 28%. Overall, N stocks in plant biomass and litter were higher in PVS compared with RNP, except in the footslope where the lower N plant stocks was associated with higher C3 abundance in RNP. Nitrogen availability was higher in CROP (25.4 ± 1.4), followed by PVS (10.2 ± 1.3), and RNP (2.2 ± 1.4); with the highest values recorded in the upslope position for PVS and RNP, and the footslope for CROP. These findings are important for designing watersheds with PVS to reduce N accumulation in the footslope position and promote additional N retention in soil organic matter and plant biomass, thereby minimizing N losses to streams.

Keywords
Agriculture, C3:C4 ratio, Carbon, N cycling, Plant litter, Plant N uptake

Disciplines
Agricultural Science | Agriculture | Agronomy and Crop Sciences | Bioresource and Agricultural Engineering | Natural Resources Management and Policy

Comments
This article is from Agriculture, Ecosystems & Environment 188 (2014): 1–11, doi:

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\textbf{A R T I C L E I N F O}

Article history:
Received 14 September 2013
Received in revised form 23 January 2014
Accepted 28 January 2014
Available online 12 March 2014

Keywords:
Agriculture
C\textsubscript{3}/C\textsubscript{4} ratio
Carbon
N cycling
Plant litter
Plant N uptake

\textbf{A B S T R A C T}

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1. Introduction

In the Midwestern U.S., conversion of native tallgrass prairie to intensively managed row-crop agriculture with high inorganic nitrogen (N) fertilizer inputs has significantly altered the N cycle (Lauenroth et al., 1999; Robertson and Vitousek, 2009). One of the major consequences of this land use change is increased N export to groundwater, streams, and rivers (Robertson and Vitousek, 2009). These N losses contribute to reduced soil fertility (Lauenroth et al., 1999) and expansion of the seasonal hypoxic zone in the Gulf of Mexico (Alexander et al., 2008; Goolsby et al., 2001; Howarth et al., 2000).

Strategies to reduce N export from agricultural lands have included the establishment of riparian buffer systems (Dosskey et al., 2002; Lovell and Sullivan, 2006) and, more recently, the incorporation of small amounts of prairie vegetation strips (PVS) in strategic locations within crop fields (Asbjørnsen et al., 2013; Helmers and Eisenhauer, 2006). Both riparian buffers and PVS function as physical barriers that reduce N losses by minimizing soil erosion (Dosskey, 2001; Dosskey et al., 2002; Helmers and Eisenhauer, 2006; Hernández-Santana et al., 2013; Zhou et al., 2010, 2014). Additionally, riparian buffers and PVS retain NO\textsubscript{3}−N through biogeochemical transformations resulting from plant uptake and microbial processes. These transformations include NO\textsubscript{3}−N transfer to above and belowground plant tissues, denitrification,
immobilization by microorganisms, leaching (Barrett and Burke, 2000; Kaye et al., 2002).

In native tallgrass prairie ecosystems, plant species composition is an important factor that can influence N and C cycling and storage, particularly due to species–specific traits associated with the C3 and C4 plant functional groups that typically dominate these ecosystems (Evink et al., 2006; Hobbie, 1992; Wedin and Tilman, 1990). For example, C4 prairie grasses typically produce more biomass (both above- and below-ground) and have more recalcitrant tissue (i.e. higher C:N and lignin:N ratios) than C3 grasses and forbs (Baer et al., 2002; Wedin and Tilman, 1990). Consequently, increasing dominance of C4 species generally decreases N and C mineralization rates (Baer et al., 2002; Epstein et al., 1998; Mahaney et al., 2008). In contrast, C3 forbs have deeper roots and take up more water and nutrients (Nippert and Knapp, 2007) and accumulate N and C deeper in the soil layers compared to C4 grasses (Fornara and Tilman, 2008). Therefore, simultaneous presence of different plant functional groups with different functional traits may sustain multiple ecosystem services in grassland ecosystems as result of niche partitioning that allow the species to capture resources in ways that are complementary in space and time (Fornara et al., 2009; Fornara and Tilman, 2008; Nippert and Knapp, 2007). Therefore, incorporation of a C3 and C4 species mix within crop fields should increase plant functional complementarity in PVS promoting retention of N from agricultural watersheds. Studies in Minnesota USA indicate high-diversity mixtures of both C3 and C4 stored 50% and 60% more soil C and N than did monoculture plots of the same species (Fornara and Tilman, 2008). Thus, mixes of C3 and C4 species in PVS within crop fields should increase SOC stocks and the accompanying stoichiometric sink for N (Barrett and Burke, 2000; Fornara and Tilman, 2008; Mahaney et al., 2008; Ramundo et al., 1999) when C accumulates more rapidly than N. Additionally, management practices such as annual burning and mowing can affect prairie vegetation composition (ratio of C3 to C4 plant species) and influence N and C cycling in RNP and PVS (Collins et al., 1998). For example, although mowing and burning influence the presence of forbs, burning declined plant richness (Carter et al., 2000). With respect to N cycling, Maron and Jeffers (2001) found substantially greater amounts of N in prairie soils with mowing, while fire reduced net N mineralization, N availability, and plant uptake, while promoting immobilization of existing N in the organic fraction of soil from turnover of organic matter of lower quality (low N content and high C:N ratio; Anderson et al., 2006; Blair, 1997; Turner et al., 1997).

Potential impacts of PVS on TN stocks and transformations in agricultural landscapes may also be influenced by landscape position. Research conducted in restored and native tallgrass prairie ecosystems showed that both N cycling and availability (Schimel et al., 1991; Turner et al., 1997), as well as plant N uptake and C storage (Knapp et al., 1993; Nippert et al., 2011; Turner et al., 1997) varied by slope position. However, knowledge about the specific mechanisms that control spatio-temporal fluxes of N and C within PVS located in different landscape positions and embedded within a matrix of annual crops is lacking (Lovell and Sullivan, 2006).

To advance understanding of the ecological functions and management of PVS with respect to N and C dynamics within agricultural landscapes, the objectives of this study were to: (1) evaluate changes in total soil organic C and N stocks under PVS compared to an annual rowcrop agricultural system (CROP) and a 17-year old reconstructed native prairie (RNP); (2) quantify the amount of N stored in plant aboveground biomass and assess the ratio of C3:C4 species within PVS and RNP; (3) determine the inorganic N availability under each of the three cover types; and (4) assess the influence of topographical position on each variable measured in #1–3 above. We hypothesized that (1) total SOC and TN stocks would be higher in PVS compared to CROP; (2) plant N stocks would be higher in PVS than RNP due to additional N supply from fertilizer applied in crop areas upslope the strips; (3) inorganic N availability would be higher in CROP compared PVS and RNP, with higher N availability in PVS than RNP because of fire management and lower C3:C4 ratios in RNP; and (4) topographical position would influence N availability and retention through redistribution of water and organic matter from upslope to downslope positions in the three covers.

2. Methods

2.1. Study area

The study was conducted at the Neal Smith National Wildlife Refuge (NSNWR), a 3000 ha area managed by the U.S. National Fish and Wildlife Service located in the Walnut Creek watershed in Jasper County, Iowa. The central mission of the NSNWR is to reconstruct the pre-settlement vegetation on the landscape, particularly native tallgrass prairie. Lands that have not yet undergone restoration activities are either maintained as pasture or leased to local farmers for production of corn and soybean using approved practices (e.g., no-till and restricted chemical inputs). Reconstructed prairies are maintained by prescribed fire, generally implemented in the spring every 1–2 years.

The NSNWR comprises part of the southern Iowa drift plain (Major Land Resource Area 108C) (USDA Natural Resources Conservation Service, 2006), which consists of steep rolling hills of Wisconsin-age loess on pre-Illinois a till (Prior, 1991). Dominant soils within the study area belong to the Ladoga series (Mollic Hapludalf), characterized as having 5–14% slopes and being highly erodible (Nestrud and Worster, 1979; Soil Survey Staff, 2003). Mean annual precipitation is 850 mm with the largest storms occurring between May and July (National Ocean and Atmospheric Administration Station at the NSNWR).

2.2. Experimental design

This study was implemented on three small adjacent watersheds (0.73, 3.0, and 0.60 ha), each of which was subjected to one of the following treatments: (1) 100% rotational row-crop agriculture of corn (Zea mays) and soybean (Glycine max; hereafter ‘CROP’), (2) 20% perennial cover (distributed in three strips: upslope, sideslope and footslope position) (hereafter ‘PVS’), and (3) 100% reconstructed 17-year old native prairie (hereafter ‘RNP’) as the control condition. In July 2007, areas receiving PVS treatment were tilled and broadcast seeded with native tallgrass prairie seed mix containing 32 species (Table 1) in a proportion of 26 C3 species (3 grasses and 23 forbs) and 6 grasses C4 (Hirsh, 2012). This mixture reflected the common mix of species used by the NSNWR staff in prairie reconstruction practices as in the RNP. The width of the PVS ranged from 27–41 m at the footslope to 5–10 m at the sideslope and upslope positions. The RNP was managed with prescribed burning in the spring every 1–2 years (April 2005, 2007, 2008, and 2010) following standard NSNWR practices. Due to logistical constraints that preclude management of PVS with fire, they were mowed annually to remove senesced vegetation. Prior to treatment, these two watersheds were dominated by perennial bromegrass (Bromus L.) for at least 10 years without fertilizer application. In August 2006, the crop areas in the watersheds and the areas to be planted to PVS were uniformly tilled with a mulch tiller. Starting in spring 2007, a 2-year no-till corn-soybean rotation (soybean in 2007) was implemented in the CROP and PVS treatments. During corn years, approximately 135 kg ha−1 of NH3-N was injected to the soil before planting.
Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andropogon gerardii</td>
<td>G</td>
</tr>
<tr>
<td>Bouteloua curtipendula</td>
<td>G</td>
</tr>
<tr>
<td>Elymus canadensis</td>
<td>G</td>
</tr>
<tr>
<td>Elymus virgincus</td>
<td>G</td>
</tr>
<tr>
<td>Schizachyrium scoparium</td>
<td>G</td>
</tr>
<tr>
<td>Sorghastrum nutans</td>
<td>G</td>
</tr>
<tr>
<td>Sporobolus spp.</td>
<td>G</td>
</tr>
<tr>
<td>Amorpha spp.</td>
<td>F</td>
</tr>
<tr>
<td>Anemone canadensis</td>
<td>F</td>
</tr>
<tr>
<td>Asteptias spp.</td>
<td>F</td>
</tr>
<tr>
<td>Aster spp.</td>
<td>F</td>
</tr>
<tr>
<td>Chamaecrista fasciculata</td>
<td>F</td>
</tr>
<tr>
<td>Coreopsis spp.</td>
<td>F</td>
</tr>
<tr>
<td>Heliotrois helianthoides</td>
<td>F</td>
</tr>
<tr>
<td>Lespedeza capitata</td>
<td>F</td>
</tr>
<tr>
<td>Liatris spp.</td>
<td>F</td>
</tr>
<tr>
<td>Monarda fistulosa</td>
<td>F</td>
</tr>
<tr>
<td>Ratibida spp.</td>
<td>F</td>
</tr>
<tr>
<td>Solidago rigidula</td>
<td>F</td>
</tr>
<tr>
<td>Ambrosia artemisiifolia</td>
<td>WF</td>
</tr>
<tr>
<td>Ambrosia trifida</td>
<td>WF</td>
</tr>
<tr>
<td>Bidens polylepis</td>
<td>WF</td>
</tr>
<tr>
<td>Brickellia rupatorioideae</td>
<td>WF</td>
</tr>
<tr>
<td>Cheno podium album</td>
<td>WF</td>
</tr>
<tr>
<td>Daucus carota</td>
<td>WF</td>
</tr>
<tr>
<td>Lactuca serriola</td>
<td>WF</td>
</tr>
<tr>
<td>Trifolium repens</td>
<td>WF</td>
</tr>
<tr>
<td>Polygonum convolvulus</td>
<td>WF</td>
</tr>
<tr>
<td>Polygonum pensylvanicum</td>
<td>WF</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>WF</td>
</tr>
<tr>
<td>Setaria faberi</td>
<td>WG</td>
</tr>
<tr>
<td>Muhlenbergia ssp.</td>
<td>WG</td>
</tr>
</tbody>
</table>

2.3. Change in SOC and TN over time

Pre-treatment soil samples were collected in the CROP and PVS watersheds in February 2005. Analysis of the pretreatment data in the same experimental watersheds, which were reported by Zhou et al. (2010), confirmed that the CROP and PVS watersheds had similar slope, soil textures, and SOC and TN concentrations. To determine SOC and TN stocks in post-treatment (2010) soils; soils cores were collected to 15 cm depth using a 6 cm diameter hand probe. Soil collection was conducted along three perpendicular transects to the watershed drainage in the PVS and CROP watersheds. 5 soil samples by topographical position (upslope, sideslope and footslope) were collected in PVS and CROP. In 2010, soil from RNP was also collected in a similar manner. Soil samples were dried and sieved with a 2.0 mm sieve. Samples were then ground in a Wiley™ mill and passed through a No. 20 stainless steel mesh (1 mm mesh size) to homogenize the sample. C and N content were determined using a Total Elemental Analyzer at the Forest Service chemistry laboratory in Grand Rapids, MN. Gravimetric moisture content was determined by oven drying a sub-sample at 105 °C for 48 h. Bulk density was used to express SOC and TN concentrations in g m⁻² in the top 15 cm.

2.4. N plant stock and functional groups in prairie vegetation

Aboveground biomass was estimated by clipping all vegetation at ground level within three 0.75 cm × 0.20 cm quadrants located in random points within each PVS. In RNP quadrants were similarly sampled along equidistant sampling points located along the same topographical positions as the PVS (e.g., footslope, sideslope, and upslope). Biomass samples were collected at three different times to represent temporal changes: July 09, August 12, and October 12. Each vegetation sample was separated into C3 and C4 functional groups. A subsample from each was further separated into live (green) and dead (litter plus standing dead) material. All samples were oven-dried at 65 °C for 48 h and weighed. Total biomass was calculated as the sum of C3 and C4 dry mass. Samples were then ground in a Wiley™ mill and homogenized by passing through a No. 20 stainless steel mesh (1 mm mesh size). N content was determined as previously described.

2.5. N availability by cover type

The ion exchange (cation and anion) resin membrane (IEM) burial method was used to assess the availability soil inorganic N among different cover types and topographical positions (Bowatte et al., 2008; Qian and Schoenau, 1994). Cation exchange membranes (CEM) sorb ammonium (NH₄⁺) and anion exchange membranes (AEM) sorb nitrate (NO₃⁻). IEMs (membrane size of 2 cm × 4 cm) were buried in the field for three separate 15-day sampling periods: June 23–July 8, August 13–28, and September 23–October 8, 2010.

Six pairs of IEMs (each pair consisting of one CEM and one AEM) were buried in the soil along three transects parallel to the drainage in each of the three topographical positions (i.e., n = 54 pairs) for each sampling period and cover type. In each transect, each of the six pairs were separated by about 20 cm, covering 100 cm of soil surface longitudinally in each transect. After 15 days in the field, IEM strips were collected, put in a cooler and transported to the laboratory where they were cleaned with deionized water to remove visible soil, the next day immediately to collection. Each IEM pair was placed in a plastic vial with 50 mL of 2.0 M KCl, shaken for 1 h, and the extracts filtered through Whatman No. 42 filter paper.

NO₃⁻ and NH₄⁺ concentrations were measured in microplates using the Griess–Ilosvay reaction with vanadium(III) chloride as a reducing agent and the Berthelot reaction, respectively (Hood–Nowotny et al., 2010). Availability of mineral soil nitrogen (NO₃⁻-N and NH₄⁺-N) under each cover type was reported in μg cm⁻² d⁻¹ by dividing the NO₃⁻-N and NH₄⁺-N concentrations (μg) by two times the membrane surface (i.e., 2 cm × 4 cm × 2 cm) and by the number of days during which each IEM was in contact with the soil. These values were used as an index of NH₄⁺-N and NO₃⁻-N availability.

2.6. Statistical analysis

The effect of PVS incorporation into crop fields was evaluated using a t-test (p ≤ 0.05) to compare total C, N, and C:N ratio of soil samples from pre-treatment (fall 2005) and post-treatment (summer 2010) periods just between PVS and CROP using the Statistical 7 software (StatSoft 1984–2004). A post hoc power analysis (p ≤ 0.05) was applied to evaluate whether any observed absence of statistically significant differences among pre- and post-treatments was due to the low replication or lack of a treatment effect (Krvachenko and Robertson, 2011). Although the watersheds (experimental unit) used in this study are typical for Central Iowa, the lack of replication at the watershed scale resulted in pseudo-replication and thus conclusions are limited to the study watersheds and cannot be generalized to the region. The effects of cover type (i.e., PVS, RNP, and CORP) and topographical position on soil parameters (SOC, TN stocks and C:N ratio) were analyzed statistically using a two-way ANOVA (p ≤ 0.05). Temporal changes in N stock in total aboveground biomass and litter by prairie cover type (PVS and RNP) were analyzed using RM-ANOVA, considering two factors: cover type (between subjects) and sampling date (within subjects). Topographical position nested within cover type was included as a third factor. Temporal changes in N stock by functional group into PVS and RNP were analyzed by RM-ANOVA with
functional group (between subjects), sampling date (within subjects), and topographical position nested within functional group as factors. The relative contribution of each functional group to the total biomass (i.e., the C$_3$ - C$_4$ ratio) was reported as the plus of three quadrant by topographical position (i.e., in 0.45 m$^2$). Temporal changes in availability of inorganic N (NH$_4$-N$_{TEM}$ and NO$_3$-N$_{TEM}$) under PVS, RNP and CROP were analyzed using repeated measures analysis of variance (RM-ANOVA). For these analyses, two factors were considered: cover type (between study watersheds) and sampling date (within study watersheds). Topographical position nested within cover type was included as a third factor. For all functional groups analyses, a post hoc multiple mean comparison test was conducted by applying a Tukey’s test with a minimum significance level of $p \leq 0.05$. Statistical analyses were conducted using the GLM procedure in SAS (SAS Institute, 2001).

3. Results

3.1. Change in SOC and TN over time

Comparing the pre-treatment and post-treatment soil properties revealed that, for CROP, TN stocks in the footslope (Fig. 1b) and C:N ratio in the upslope (Fig. 1c; Table 2) decreased, while SOC did not show significant changes. In PVS, SOC and TN stocks increased significantly in the footslope, while the soil C:N ratio in the footslope decreased (Fig. 1c; Table 2).

Comparing soil properties between the three cover types and topographical positions from 2010, cover type affected SOC ($p = 0.0109$) and TN ($p = 0.0286$) stocks, while topographical position ($p < 0.0001$) and its interaction with cover type ($p < 0.0001$) had higher influence the C:N ratio. For both PVS and RNP, SOC (Fig. 2A) and TN stocks were highest in the footslope position (Fig. 2B). PVS had significantly higher SOC in the side and footslope positions relative to RNP (Fig. 2A), while TN stocks were higher for PVS than RNP across all topographical positions (Fig. 2B). In contrast, for CROP, SOC and TN stocks were highest for upslope and lowest for footslope, although these differences were only significant for soil N (Fig. 2B).

Soil C:N ratio (Fig. 2C) was related to topographical position ($p < 0.0001$) and the interaction between topographical position and cover type ($p < 0.0001$). Comparing across cover types, soils under RNP showed a declining trend in the C:N ratio from upslope to footslope, while the opposite pattern was observed for soils under CROP (Fig. 2C). No significant differences were observed for the soil C:N ratio between the three topographical positions for PVS.

3.2. Aboveground plant N stocks and prairie functional groups

The results indicated that within each cover type, aboveground plant N stocks varied with topographical position ($p = 0.0270$), while no differences were observed when comparing between cover types ($p = 0.2090$). For PVS (Fig. 3a), the highest plant N stocks were recorded in October in plants growing in the sideslope position,
Table 2
Results from t test (α = 0.05) at the comparison of total C, N and C:N ratio between pretreatment (pre-treatment; early 2005) and post-treatment (June, 2010) by topographical position into CROP and PVS.

<table>
<thead>
<tr>
<th>Cover type</th>
<th>Topographical position</th>
<th>p value t test</th>
<th>Sample size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CROP</td>
<td>Upslope</td>
<td>0.890</td>
<td>4</td>
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<tr>
<td></td>
<td>SOC</td>
<td>0.059</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>TN</td>
<td>0.014</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>C:N ratio</td>
<td>0.710</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>SOC</td>
<td>0.328</td>
<td>4</td>
</tr>
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<td></td>
<td>TN</td>
<td>0.140</td>
<td>4</td>
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<tr>
<td></td>
<td>C:N ratio</td>
<td>0.084</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>SOC</td>
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<tr>
<td></td>
<td>TN</td>
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<tr>
<td></td>
<td>C:N ratio</td>
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<tr>
<td>PVS</td>
<td>Upslope</td>
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<td></td>
<td>SOC</td>
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<tr>
<td></td>
<td>TN</td>
<td>0.072</td>
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<tr>
<td></td>
<td>C:N ratio</td>
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<td></td>
<td>SOC</td>
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<td></td>
<td>TN</td>
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<td>0.005</td>
<td>4</td>
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Note: Only CROP and PVS were included in this comparison because we did not sample the RNP treatment in 2005, due to administrative restrictions. SOC: soil organic carbon.

and these values were significantly different (p < 0.05) from those observed for plants growing in the footslope position but not the upslope position. Litter N stock in PVS varied across sampling times (p = 0.0063) and marginally by cover type, with significantly higher litter N recorded in October (Fig. 3d) and for the sideslope position. Within RNP, vegetation in the footslope generally exhibited higher N stocks compared with upslope and sideslope positions (Fig. 3c); but these differences were only significant in July and October. Litter N stocks for RNP did not show spatial or temporal differences (Fig. 3d).

When the plant N stock data were explored in greater depth, results showed a trend of higher N stocks for PVS than RNP across all functional groups and topographical positions (Fig. 4). Further, C3 grasses generally had lower N stocks compared to C4 species in both PVS and RNP (Fig. 4). No temporal or topographical differences were observed in plant N stocks by functional group. Results from the RM-ANOVA were only significant at the general level of functional groups (p = 0.0063). No C3 grasses were recorded in the footslope position for PVS in July (Fig. 4a) or for RNP in August and October (Fig. 4d), while C3 grasses were not recorded in the sideslope position in August and October (Fig. 4e).

The C3:C4 ratio was highly variable temporally and spatially between and within PVS (Fig. 5a–c). In RNP (Fig. 5d–f), a clear opposite pattern in the C3:C4 ratio was observed in footslope (lowest biomass of C4 species) and sideslope (lowest biomass of C3 species). In PVS, highest and lowest C3 (grasses and forbs): C4 ratio were in footslope (50.48) and sideslope (1.72) positions, respectively; C1 (grasses): C3 ratio was highest in upslope during July (4.31) and lowest in sideslope during October (0.02), and finally C1 (forbs): C3 ratio was highest in upslope during July (21.37) and lowest in sideslope positions during October (0.80). Within PVS, C3 plant biomass was relatively high across all sampling times and topographical positions compared to RNP, which had relatively low C3:C4 ratios during the entire study period but only in the upslope and sideslope positions. The footslope position of RNP, particularly forbs was the dominant functional group in August (Fig. 5e) and October (Fig. 5f). Therefore, when comparing between RNP and PVS, biomass of C3 grasses was the dominant functional group for PVS (Fig. 5a–c), while in RNP the dominance of each functional group depended on the topographical position (Fig. 5d–f).

3.3. N availability by cover type

Cover type, time, and their interaction had a significant (p < 0.05) effect on NH4 -NCEM availability (Table 3), but topographical position nested within cover type and the time × cover type interaction did not influence NH4 -NCEM availability. Conversely, the NO3 -NCEM availability was affected (p < 0.05) by all factors analyzed (i.e. cover type, time, topographical position nested within cover type) and their interactions (Table 3), with a similar pattern observed for total inorganic N availability (NH4 -NCEM + NO3 -NCEM). Analyzing by cover type, RNP showed higher (p < 0.05) average NH4 -NCEM (1.9 ± 0.3) compared to PVS (0.7 ± 0.3) and CROP (0.3 ± 0.3). Temporal differences were observed for RNP, with significantly higher NH4 -NCEM occurring in October–November (Fig. 6c) when differences between topographical positions were due to low NH4 -NCEM rates in the footslope. For PVS and CROP, no significant differences were observed for NH4 -NCEM among the different topographical positions or sampling times (Fig. 6a and b).

With respect to NO3 -NCEM (Fig. 6d), CROP (25.1 ± 1.4; Fig. 6d) showed the highest values, followed by PVS (9.5 ± 1.2; Fig. 6e), while the lowest values were recorded for RNP (0.3 ± 1.4; Fig. 6f). Only PVS and CROP showed temporal differences, with
significantly higher NO$_3$-N$_{_{\text{AM}}}$ in October compared to the two earlier time periods. For PVS, differences were observed between topographical positions for NO$_3$-N$_{_{\text{AM}}}$ in August and October (Fig. 3c, d), with higher values for the upslope position during both time periods. For CROP (Fig. 3e), topographical position also showed differences in August and October-November, with significant higher values of NO$_3$-N$_{_{\text{AM}}}$ recorded at the footslope position.

### 4. Discussion

This study showed that the incorporation of PVS in rowcrop agricultural systems has the potential to increase SOC and TN stocks in the Midwestern region of the U.S. Increments varied spatially across topographical positions with higher soil C and N accumulation under PVS located in the footslope position relative to upslope or sideslope positions. Although a conclusive relationship between plant N uptake by functional group and total plant N uptake could not be established due to high variability of species composition under field conditions, here trends suggest that the higher plant N stocks and biomass observed of C$_3$ vegetation may have contributed to greater retention of fertilizer N by PVS than C$_4$ species.

#### 4.1. Retention of N and SOC at PVS sites

After 3 years of PVS establishment, soils under PVS experienced a 37% increase in SOC and almost 100% increase in TN stocks in the footslope position, resulting in significantly lower C:N ratios compared to pre-treatment levels. In contrast, in the CROP watershed, SOC and TN stocks decreased in the footslope position (28% and 31%, respectively). Therefore, the incorporation of PVS within crop fields should increase SOC stocks, which contribute to enhancing soil N retention (Drinkwater and Snapp, 2007). These findings are consistent with our first hypothesis, which predicted higher soil TN and SOC stocks in PVS compared to CROP by the third year following PVS establishment.

Prairie vegetation within PVS and RPN likely promote N retention and stabilization in the soil through two primary mechanisms: (1) plant uptake that results in the incorporation of N into the above and belowground plant biomass (Aber et al., 1998); and (2) microbial immobilization and subsequent transfer to soil organic matter (Davidson et al., 1991). These mechanisms are influenced by management practices related to the location and composition of PVS. Accordingly, when aboveground biomass is removed during annual mowing, roots from different functional groups represent an active soil N pool that may sustain the N plant uptake during the next regrowth period. Additionally, the C pool also increased following mowing with the presence of different functional groups, likely due to greater complementary in the use and demand of resources associated with varying intrinsic root system traits (Fornara and Tilman, 2008; Fornara et al., 2009).

#### 4.2. N soil retention and plant N stock at PVS

Plant uptake controls N stocks in plant tissue, which in turn depends on soil nutrient availability (Iversen et al., 2010; Wedin
and Tilman, 1990). In this study, despite differences in aboveground biomass, N plant stock was not significantly different between cover types (p = 0.2090). There was a trend of spatially (across topographical positions) and temporally (across three sampling times) higher plant N stocks in PVS when compared to RNP. Additionally, the higher N stock in PVS vegetation was consistent with a higher litter N stock, indicating higher N availability in PVS. Further, similar patterns in NO3-N availability were found in CROP as in PVS, while the opposite pattern found in RNP suggests the transference of N fertilizer applied in crop areas to the PVS, as a result of downslope runoff interaction in the mixed watersheds (Zhou et al., 2010). These findings are in agreement with our second hypothesis, which predicted higher plant N stocks in PVS than RNP due to additional N supply from fertilizer applied in crop areas upslope of the strips.

Regarding our third hypothesis, results in this study showed effectively higher N availability (NO3-N plus NH4-N) in CROP compared to areas with prairie vegetation (PVS and RNP), and consequently also higher N availability was found in PVS compared to RNP, due to fertilizer applied in the crop areas where the strips were incorporated. However, the lack of an association between higher N availability and SOC stocks in soils under CROP, together with the findings supporting our second hypothesis (above), suggest that the source of that inorganic N was the supplied fertilizer in crop areas and not only the in situ soil pools.

Plant N stocks in PVS and RNP may also reflect differences in plant N uptake between functional groups due to generally lower demand of N from C4 grasses compared to C3 forbs and grasses (Seastedt et al., 1991; Wang et al., 2008). In this study, lower N stocks in vegetative tissue from C4 grasses compared to C3 grasses and forbs confirmed these patterns. Further, the greater C3:C4 ratio (mainly attributed to C3 forbs) in PVS together with higher plant N stocks supports the notion of greater N uptake as the proportion of C3 grasses increases. Additionally, because PVS allows for litter inputs throughout the sampling times due to temporal changes in plant composition, these inputs may promote continuous renewal of the organic matter pool and, consequently, sustained abiotic N fixation. Notably, in this study, the higher litter N stock in PVS was likely affected by the higher N availability from fertilizer use, indicating that the N returned to the soil via litter production would also increase (Huang et al., 2008; Kobe et al., 2005; Ratnam et al., 2008), further stimulating chemical N fixation in soil organic matter, the most stable pool of soil N. Thus, litter N stocks could provide a useful indicator of biotic and abiotic incorporation of N into PVS.

**Fig. 4.** N plant stock in biomass from C4 grasses, and C3 grasses and forbs within Prairie Vegetation Strips (PVS; a, b and c); and from Reconstructed Native Prairie (RNP; d, e and f, respectively).
4.3. Influence of management tools in N retention at PVS

Long-term N fertilization can directly influence plant N dynamics and competitive interactions (Baer et al., 2002; Huang et al., 2008; Wedin and Tilman, 1990); and consequently, the natural spatial and temporal changes in the C4:C3 ratio of the PVS. Huang et al. (2008) conducted a 3-year experiment to determine the effects of N fertilization on N resorption of six temperate grassland species belonging to different life forms (grass, semi-shrub, N-fixer, and forbs). Findings indicated that long-term N fertilization had differential effects as increased plant N uptake and decreased plant N resorption proficiency, depending on the species. Therefore, litter quality and its decomposition, which are largely determined by lignin:N ratio, depends on species composition and controls the rate at which organic matter is mineralized over short and long time periods (Baer et al., 2002; Wedin and Tilman, 1990). The increment of N availability because of supplied fertilizer also could induce differences in species composition (C4:C3 ratio) into the PVS. For instance, Seastedt et al. (1991) reported that fertilizer application in tallgrass prairie resulted in an increase in forb biomass and a decrease in grass biomass. These findings are consistent with our findings suggesting the greater effectiveness of C3 species in incorporating N from fertilizer into the internal cycle allowing a faster incorporation of N into high quality organic matter into the PVS.

Regarding mowing and spring burning, these stimulate plant productivity of prairie vegetation, influence biotic N incorporation into vegetative tissue of plants, and alter species composition (Carter et al., 2000; Turner et al., 1997). Thus, these two common restoration tools may increase the capacity of PVS to take up additional N from fertilizer applied into the crop areas between the strips. Plant productivity is stimulated because fires remove the plant litter on the soil surface (Knapp and Seastedt, 1986), and both fire and mowing remove aboveground biomass creating more favorable light conditions leading to increased photosynthetic activity and plant growth (Briggs and Knapp, 1995; Turner et al., 1997), and hence, higher plant N uptake. Frequent fire increases the dominance of perennial C4 grasses while reducing abundance and productivity of C3 grasses, forbs and woody plants (Collins et al., 1998; Lauenroth et al., 1999; Turner et al., 1997). The increase of the C4 proportion in the burned tallgrass prairie was confirmed in this study with lower C3:C4 biomass ratios in RNP (Fig. 6). Additionally, N cycling is influenced by repeated annual
burning through the production of greater inputs of lower quality plant residues and a significant reduction in soil organic N, lower N availability, and higher C:N ratio in soil organic matter (Ojima et al., 1994). Mowing, however, influences N cycling by promoting plant diversity and the presence of forbs (Carter et al., 2000; Collins et al., 1998). Fire and mowing can independently influence ecological processes in grasslands, and they may be having important interactive effects, as seen for fire and grazing (Hobbs et al., 1991). Collins et al. (1998) found that richness declined on burned and fertilized treatments, while mowing maintained diversity under the same conditions (Collins et al., 1998). In this study, lower C3:C4 ratios were found in RNP managed with periodical burnings, and higher C3:C4 ratios were found in PVS that were influenced by both fertilizer inputs and mowing. Therefore, our third hypothesis was supported with respect to the higher N availability in PVS when compared with RNP, in part because of fire management and lower C3:C4 ratios leading to lower N availability in the latter.

4.4. Topographical position and N retention effectiveness at the PVS

As mentioned earlier, topographical position influenced N transformations, redistribution and retention within the three cover types, lending support to our fourth hypothesis where we expected
an influence of topographical position on N availability and retention through spatial redistribution of water and organic matter from upslope to downslope positions in the three watersheds. Here, topography also influenced the temporal response of NO$_2$-N, with higher amounts of NO$_2$-N in the footslope position observed in the CROP in August and October. This is consistent with a downslope flowpath of NO$_2$-N reported by Zhou et al. (2010) for studies in some of the same watersheds. This increment occurred despite a concomitant increase in the downslope stoichiometric sink for N (increasing downslope C:N ratios). Thus, the more mobile NO$_3^-$ is more easily transported by downslope flow during rainfall events, leading to subsequent losses of NO$_3^-$ by leaching at the footslope (high soil C:N ratios). According to Aber et al. (1998), C:N ratio is typically negatively correlated with N leaching losses, reflecting both past land use and current N status.

Further, the inconsistency between higher N in plant biomass and litter in the sideslope PVS without a concomitant increase in SOC, combined with the higher TN stock, SOC (Fig. 2) and NO$_3^-$ N$_{AEM}$ observed in the footslope, suggest that in PVS, plant N uptake is the dominant mechanism of N retention in the sideslope but not in the footslope position. This is also in agreement with the downslope NO$_3^-$N transport suggested by Zhou et al. (2010) and the efficacy of downslope NO$_3^-$N removal by PVS. Saturated conditions in the footslope might promote NO$_3^-$N$_{AEM}$ accumulation by the presence of microorganisms and NO$_3^-$N$_{AEM}$ losses by leaching and denitrification.

5. Conclusions

In this study, while annual rowcrop systems become progressively C and N depleted, PVS accumulated soil C and N over time showing high effectiveness in improving N cycling and retention functions after only 3 years following establishment. Results indicate that N retention into PVS is the result of many simultaneous biological and geochemical mechanisms. Although a conclusive relationship between plant N uptake by functional group and total plant N uptake could not be established, trends suggest that the higher plant N stocks observed for C$_3$ species and its higher biomass could contribute to greater retention in PVS of N from fertilizer. Results also showed how topographical position influenced PVS SOC and TN stocks distribution and retention effectiveness. Thus, the main mechanism to retain N depends on the PVS location within the watershed, with plant N uptake dominating in the sideslope position, and accumulation and chemical fixation dominating in the footslope position.

Our findings regarding the accumulation of TN and SOC stocks in PVS generates new questions regarding the ecological and geochemical mechanisms that control N recycling and losses in PVS. For example, to what extent do C$_3$ and C$_4$ prairie species and annual C$_4$ crop species differ in their above and belowground biomass production and allocation to different plant components (stem, leaves and roots)? Further, what is the influence of C$_3$ and C$_4$ species on N mineralization and N availability under conditions that include N fertilization? To understand the enhanced role of PVS to reduce N (NO$_2$ and NO$_3^-$) losses from agricultural watersheds, we suggest evaluating the N uptake and allocation by C$_3$ and C$_4$ species under field and experimental conditions utilizing fertilizer labeled with $^{15}$N to elucidate the particular effects of plant functional groups on N cycling.

Acknowledgements

The authors gratefully acknowledge the support of Pauline Drobnay and the staff at the Neal Smith National Wildlife Refuge. Thanks to Christopher Witte for his assistance on field work.

Funding for this project was in part provided by USDA Grant 2010-85101-20469.

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