Nitrogen dynamics in grain cropping systems integrating multiple ecologically based management strategies

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Abstract. Nitrogen (N) management is a critical agronomic challenge, as N losses are a source of pollution affecting both waterways and air quality and a potential economic loss for farmers. One approach to N conservation is through ecologically based agricultural systems that reduce tillage and incorporate cover crops. However, these systems exhibit considerable complexity resulting in potential agronomic trade-offs. To address these concerns, four crop management systems were implemented within an organically managed corn–soy–winter grain crop rotation. These systems varied in tillage frequency and intensity, cover crop species selection, cover crop termination and establishment methods, fertilizer management, and cash crop season length. We used field measurements to investigate the impact of each system on N pools and to reveal the strengths and weaknesses of each system in addressing N provisioning services, with a focus on the supply and retention of N before and after the corn phase of the rotation. All systems had greater estimated N inputs (via manure and N-fixation) than outputs (via crop harvest) at the end of the three-year rotation, demonstrating the importance of prioritizing N retention in cover crops. Interactions among system components were important drivers of temporal N dynamics; cover crop species traits and timing of manure application contributed to differences in total aboveground plant biomass N among systems. For example, one cropping system which included a no-till corn planting into a rolled cover crop mulch had soil inorganic N availability that was asynchronous with the N needs of the corn crop even though it received the same amount of N inputs as the other systems. In general, neither interseeding cover crop mixtures nor reducing tillage resulted in marked N benefits at the system level; we did not observe improved N retention from either practice in these systems, and there was no increase in N uptake by corn. What did clearly emerge from this experiment is the importance of managing for synchrony between soil inorganic N availability and cash crop N demand as influenced by the N retention capacity of cover crops and the timing of N mineralization due to tillage.

Key words: agroecosystems; conservation tillage; corn; cover crops; cropping systems; interseeding; nitrogen; organic agriculture; reduced-tillage; soils; sustainable agriculture.

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INTRODUCTION

Ecologically based agriculture can reduce negative impacts on the environment while also directly benefiting farmers (Foley et al. 2011, Schipanski et al. 2016, LaCanne and Lundgren 2018). For example, implementing a reduced-tillage regime by reducing the intensity or frequency of tillage events will minimize soil disturbance and provide soil health benefits (Berner et al. 2008). Employing organic agriculture is another example, which eliminates synthetic inputs and relies on nutrient recycling through organic inputs that couple carbon (C) and nitrogen (N). Another ecologically based strategy is integrating cover crops, which are planted for other benefits rather than for harvest. Cover crops can increase plant cover spatially and temporally, take advantage of resource partitioning for nutrient retention and recycling, fix N, and add plant diversity. However, it is a challenge to integrate and implement multiple strategies in one agroecosystem.

Without the option to use synthetic herbicides, organic agriculture has traditionally relied heavily on intensive tillage to manage organic inputs and control weeds, a strategy with environmental and agronomic trade-offs (Follett 2001, Carr et al. 2006, Teasdale et al. 2007). However, managing organic agricultural systems without intensive tillage, especially those that incorporate high-biomass cover crops, poses a challenge in predicting and controlling essential N services (Cavigelli et al. 2013, Keene et al. 2017). Designing organic cropping systems to mitigate N losses while maintaining crop yields has direct links to agricultural sustainability: the reduction in environmental risks associated with nitrate leaching into the water supply, the enhancement of economic productivity for farmers, the conservation of soil, the improvement of water quality, and the protection of natural resources (Robertson and Vitousek 2009, Davidson et al. 2011, Foley et al. 2011).

In highly managed agroecosystems with significant N removal through crop harvest, the soil N pool rapidly depletes and must be replenished in order to maintain crop yields (Robertson and Vitousek 2009). Managing these necessary N additions in organic agroecosystems is particularly difficult as biological processes required to mineralize organic N additions are influenced by multiple factors, including temperature, moisture, relative C and N content and N:lignin ratio of incorporated organic inputs (Nicolardot et al. 2001, Drinkwater and Snapp 2007, Blesh and Drinkwater 2013, Finney et al. 2015). Annual cropping systems, as opposed to perennial systems, are positioned for even greater N losses through soil water via leaching because of lack of established plant cover throughout the year leading to limited N uptake (Drinkwater and Snapp 2007). Temporal synchrony between plant N demand and soil inorganic N availability is essential to minimize N movement into other ecosystems while maximizing aboveground biomass production and yields (Cook et al. 2010). Incorporating overwintering plant species, planting mixtures of species to include multiple plant functional groups, and reducing the frequency and intensity of disturbances are all strategies to improve N retention and provisioning in agroecosystems.

Attention to timing and plant species selection is important when integrating cover crops into an agroecosystem. Filling otherwise empty spatial and temporal niches with cover crops selected for specific plant species traits can provide maximum benefits and prevent negative impacts on cash crop performance (Snapp et al. 2005, King and Blesh 2018). In annual cropping systems, establishing plant cover year-round is a strategy to reduce N losses through leaching; however, a limited window for fall plant establishment and growth can pose a challenge to this strategy. One solution to this constraint is to interseed cover crops directly into a standing cash crop to extend the cover crop growing season. This strategy takes advantage of resource partitioning in the spatial niche between established cash crop rows and provides a longer cover crop growing season with potential to increase N retention as well as weed suppression (although competition with the cash crop is a consideration; Abdin et al. 1998, Grabber et al. 2014, Sandler et al. 2015, Blanco-Canqui et al. 2017, Bybee-Finley et al. 2018, Curran et al. 2018, Noland et al. 2018, Wallace et al. 2020). Additionally, inherent N-fixing and N-scavenging traits of known agronomic cultivars can be used to target N services in the crop rotation (Kaye et al. 2019). For example, a high-biomass grass cover crop...
may be used to scavenge and retain soil N after a cash crop harvest while a legume cover crop may be used to provide an additional source of fertility in the system before cash crop planting. Mixtures of species with different functions can be used to affect multiple N services simultaneously (Finney et al. 2017). High-biomass cover crops can also be terminated using a roller-crimper and then left on the soil surface to act as a mulch, providing moisture retention and weed suppression benefits but with varying impacts on soil nutrient availability (Mischler et al. 2010, Parr et al. 2011).

While organic systems incorporate many practices that promote soil building and environmental protection, there is still a need for reducing side effects that come from reliance on high-disturbance tillage (Peigné et al. 2007, Armengot et al. 2015, Seitz et al. 2019). Tillage can degrade soil structure, impact water penetration, and decrease soil microbial biomass, and can lead to increased potential for erosion and depletion of soil organic matter (Mikha and Rice 2004, Teasdale et al. 2007). Tillage events stimulate N mineralization rates, often at times asynchronous with crop N demand leaving nitrate in the soil susceptible to leaching (Calderón et al. 2001, Finney et al. 2015). An improved understanding of soil N-cycling in organic reduced-tillage systems is needed both to improve fertility management strategies and to assess the environmental impacts of these systems.

In this experiment, four management systems were designed within a corn–soy–winter grain crop rotation, which is typical of an organic grain operation in the northeastern USA. These experimental systems vary in tillage frequency and intensity, cover crop species selection, cover crop termination and establishment methods, and cash crop season length. We used field measurements to investigate the impact of each system on N pools and to reveal the strengths and weaknesses of each system in addressing N provisioning services, with a focus on the supply and retention of N before and after the corn phase of the rotation. While we expect that these alternative system components may be successful in targeting N provisioning, they might also result in other agronomic challenges, benefits, or management trade-offs within different phases of the rotation. Other studies have focused on the mechanistic understanding of a single agronomic practice in isolation that would be a component of such a system (Liebman et al. 2018, Noland et al. 2018, Ruark et al. 2018), but this experiment aims to evaluate cropping systems as a whole. Systems experiments such as this allow us to evaluate emergent properties that develop from integrated component practices, moving from “simplistic cause-and-effect relationships, to a holistic view that encompasses all parts of the system and the interconnectedness among those parts” (Drinkwater et al. 2016). This approach is especially useful when integrating several ecologically based agricultural practices that may interact in complex ways that are difficult, or even impossible, to isolate in factorial experimental designs.

**METHODS**

**Site description and experimental design**

This systems experiment was conducted at the Russell E. Larson Agricultural Research Center in central Pennsylvania (40°43’ N, 77°55’ W) in 2014–2018. The soil is a well-drained Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalf). This research center is in Zone 6b of the USDA Plant Hardiness Zones. Daily temperature, precipitation, solar radiation, wind speed, and relative humidity based on satellite observations were obtained from Phase 2 of the North American Land Data Assimilation System (Xia et al. 2012). This site has an average annual rainfall and snowfall of 1006 and 1140 mm, and average monthly temperature range of −3°C to 22°C (Xia et al. 2012). Average annual potential evapotranspiration for 2015–2017 was calculated with the Penman-Monteith equation to be 1056 mm (Xia et al. 2012). Monthly precipitation data during the growing season over the course of the experiment were obtained from the Natural Resources Conservation Service (NRCS) Rock Springs, Pennsylvania, Weather Station (Appendix S1: Table S1).

This was a replicated, randomized complete block experiment with a three-year corn (Zea mays L.) to soybean (Glycine max L. Merr.) to spelt (Triticum spelta L.) rotation. Each of the four blocks contained three main plots (110 × 18 m) that were randomly assigned to one starting crop to begin the rotation. Each block contained one...
corn start, one soybean start, and one spelt start. These starts will be referred to by the year of corn planting (e.g., “2015 start” for corn planted in 2015). Each main plot was divided into four cropping system treatment subplots (49 × 9 m). The land maintained organic certification throughout the course of the experiment.

**Cropping system treatments**

Four unique cropping systems were designed to integrate ecologically based components that included reduced-tillage and innovative cover cropping strategies. These systems varied in tillage frequency and intensity, cover crop species selection, cover crop termination and establishment methods, fertilizer management, and cash crop season length. These components and strategies are described in Table 1. A full description of each cropping system design—how all components fit together—is shown in Fig. 1. A list of all operations in each system and cash crop phase is included in Appendix S1: Table S2. Crop varieties and seeding rates are included in Appendix S1: Table S3.

Many management considerations were included in the design of these systems, including nutrient management, weed management, pest and disease mitigation, and operational costs. Each system received equal amounts of fertilizer in the form of liquid dairy manure over the course of the three-year rotation. Liquid dairy manure was added in each system before spelt planting (252 kg N/ha) and before corn planting (304 kg N/ha). These rates were determined based on annual soil tests, predicted P removal rates, and the Penn State Agronomy guide. These rates are similar to what farmers operating an organic grain farm in the northeast would apply. It is important to note that these rates are most likely greater than what could be generated from animal waste raised on crops supplied by these systems, speaking to the larger picture of sustainability within organic grain cropping systems in this region. Each system also integrated one overwintering legume cover crop. We predicted that although these systems received comparable levels of N inputs, systems would differ in N retention and losses based on the interactions of management strategies applied.

System 1 (S1) represented the standard practice for rotational organic no-till grain (Mirskey et al. 2012, Wallace et al. 2017). Corn grown for silage was no-till planted into a rolled-down hairy vetch + triticale cover crop mixture. Soybean was no-till planted into a rolled-down cereal rye cover crop. Inversion tillage with a moldboard plow, which is a high-intensity tillage event, occurred after each cash crop harvest to establish winter spelt and cover crops, resulting in three high-intensity inversion tillage events over the three-year rotation. Manure was applied and incorporated into the soil before spelt planting and again before the fall seeding of the hairy vetch + triticale cover crop mixture. We predicted that this system may have asynchronous N availability during corn crop growth, leading to reduced N uptake by the cash crop and increased N losses from the system.

System 2 (S2) aimed to overcome the predicted limitations of S1 by introducing an alternative approach to managing high-biomass cover crops before corn and soybean. Like in S1, a hairy vetch + triticale mixture was grown before corn; however, it was terminated with inversion tillage instead of being rolled down. This strategy allowed for earlier corn establishment; corn grown for grain, instead of silage, was seeded about 2 weeks earlier in S2 than in S1. We predicted that this would increase the synchrony of N availability with corn growth, potentially reducing N losses during this time and increasing potential for N uptake by the corn. An annual ryegrass + orchard grass + forage radish cover crop mixture was interseeded into the established corn. This eliminated the need for a fall tillage event after corn harvest. S2 had two minimum tillage events (chisel plowing, which is non-inversion tillage) and two high-intensity tillage events (inversion tillage with moldboard plowing). Manure was applied and incorporated before winter spelt planting and before corn planting in the spring. We expected this system to result in higher N uptake by corn and higher N retention than S1.

System 3 (S3) further reduced tillage intensity by replacing the hairy vetch + triticale cover crop mixture in S1 and S2 with a no-till red clover + timothy cover crop mixture before corn grown for silage. This mixture was no-till drill seeded into spelt in late winter, a method called frost seeding. Underseeing red clover in late winter or spring into winter grains has been shown
to provide multiple benefits over hairy vetch (Snyder et al. 2016), including that red clover can produce an equivalent or greater amount of N compared with hairy vetch because of a lengthened growing season provided by frost seeding. As in S1, soybean was no-till planted into a rolled cereal rye cover crop. As in S2, termination of the cover crop mixture before corn by tillage allowed for earlier corn planting, and therefore earlier silage harvest and earlier cereal rye establishment after corn—all components that we predicted would increase N retention in this system compared to S1 and S2. S3 had one minimum tillage event (chisel plowing) and two high-intensity tillage events (moldboard plowing). Manure was applied and incorporated before winter spelt and spring corn planting.

System 4 (S4) was designed to be the lowest tillage system by combining the alternative approaches in S2 and S3 for reducing tillage: interseeding annual ryegrass + forage radish into standing corn and no-till drill frost seeding red clover + timothy into spelt in late winter. Also, before spelt planting, manure was injected using sub-surface banding technology (Maguire et al. 2011), then spelt was no-till drill seeded into soybean residue, eliminating a fall tillage event. S4 had only two high-intensity tillage events. Manure was also applied and incorporated before corn planting. We predicted that this system would perform the best in terms of N retention as it incorporated the least intensive tillage regime and integrated interseeded cover crops, no-till planting of a cash crop, and sub-surface application of manure.

### Table 1. Tillage and cover cropping strategies included in an organic grain cropping systems experiment.

| Strategy                          | Components                                      | Expected outcome                                      | System | No. occurrences (crop) |
|-----------------------------------|-------------------------------------------------|-------------------------------------------------------|--------|------------------------|
| **Tillage: reduce disturbance intensity** | Replace moldboard plowing with chisel plowing to reduce soil mixing | Comparable SIN availability | S2 3  S3 1  S4 1 |
|                                   | Replace plowing in manure with sub-surface manure injection | Greater N uptake, greater N retention | S4 1 |
| **Tillage: reduce disturbance frequency** | Eliminate tillage event by no-till seeding a cash crop directly into terminated cover crop mulch | SIN availability asynchronous with demand and insufficient to meet N demand of non-legume cash crop | S1 2 (corn, soy)  S3 1 (soy) |
|                                   | Eliminate tillage event by no-till seeding a cover crop into standing cash crop (i.e., interseeding) | Greater N retention, less SIN availability | S2 1 (into corn)  S3 1 (into spelt)  S4 1 (into spelt, corn) |
| **CC: fill fallow niches**       | Establish cover crop by no-till seeding into standing cash crop (i.e., interseeding) | Greater N retention, less SIN availability | S2 1 (into corn)  S3 1 (into spelt)  S4 1 (into spelt, corn) |
| **CC: target nitrogen scavenging, retention, and supply** | Scavenge and retain N with a grass or brassica CC | Greater scavenging in fall with brassica, less retention through spring, greatest retention with high-biomass grass | S1 2 (grasses)  S2 3 (grasses) + 1 (brassica)  S3 2 (grasses)  S4 3 (grasses) + 1 (brassica) |
|                                   | Supply N with a legume CC                        | Comparable N provisioning with hairy vetch and frost seeded red clover | S1 1 (hairy vetch)  S2 1 (hairy vetch)  S3 1 (red clover)  S4 1 (red clover) |
| **CC: incorporate multiple nitrogen services** | Replace a single species with a mixture of CCs | Greater N-scavenging, retention, and less N loss | S1 1 (before corn)  S2 2 (before and after corn)  S3 1 (before corn)  S4 2 (before and after corn) |

**Notes:** Row headings describe the ecological strategy being tested, including tillage related strategies and cover cropping (CC) strategies. The second column lists components of the agroecosystem that target each strategy. Expected outcomes are in terms of nitrogen (N) cycling and soil inorganic nitrogen (SIN) availability. The last two columns indicate the experimental systems (S1–S4) implementing each component, the number of occurrences of each component within that system, and the crop it pertains to.
Assessment of nitrogen dynamics

Extractable soil inorganic N (SIN) concentrations were measured in surface soils throughout the experiment during the growing season. SIN represents the sum of extractable ammonium N and extractable nitrate N. Soil samples were collected from each plot by compositing six 0.20 m depth by 0.02 m diameter cores. Additionally, deep soil cores were collected at the end of the experiment on 1 June 2018 from the 2017 start, which were plots in the post-corn phase of the crop rotation. Two cores were collected from each plot to 0.80 m depth and separated into 0.20 m increments for analysis. After homogenizing each soil sample, fresh 20 g subsamples were extracted for inorganic nitrogen with 100 mL of 2 mol/L KCl. Following 1 h of shaking, extracts were filtered through Whatman Grade 1 filter paper (GE Healthcare Life Sciences, Buckinghamshire, UK) and frozen until further analysis. Extracts were analyzed colorimetrically for ammonium (NH$_4^+$) and nitrate (NO$_3^-$) concentrations (mg N/kg soil; Sims et al. 1995, Doane and Horwáth 2003). Additional fresh 10 g subsamples were dried at 105°C for 48 h and weighed to calculate gravimetric water content. Rock content was estimated by wet sieving each sample with a 2-mm mesh sieve, considering particles larger than 2 mm as rocks. Sample weight was corrected for soil gravimetric water content and rock content. Manure samples were sent to the Agricultural Services Analytical Laboratory at The Pennsylvania State University for nutrient analysis.

Cover crop aboveground biomass N content was measured in the fall prior to the first hard frost between late October and early November, and in spring prior to cover crop termination in mid- to late-May. We clipped plant biomass at the surface of the ground from six to nine randomly distributed 0.25-m$^2$ quadrats in each plot. Samples were sorted by species, and plant material was dried for at least five days at 65°C. After weighing the total biomass of each species from each plot, three cover crop subsamples per plot were composited by species and ground to a fine powder. A subsample of this powder was rolled into a tin capsule and analyzed for C and N contents by dry combustion analysis (CHNS-O CE Instruments Thermo Electron Corp Elemental).
Analyzer EA 1110 with thermal conductivity detector; Matejovic 1996). Subsamples of cash crops (corn silage, corn grain, soybeans, and spelt grain) were also analyzed as above for C and N contents. Additionally, corn population stand was assessed in late August by counting the number of plants in a 5.3-m row from three random locations in each plot.

Nitrogen inputs and exports were estimated for each system within each start. This was done to estimate a partial N mass balance focused on N pools that were directly managed in these systems. N inputs were estimated as total N from manure additions plus legume cover crops, and exports were estimated as total cash crop N removed. Legume cover crop contribution was calculated for each plot based on the %N content of total aboveground legume biomass. This method overestimates the contribution of new N to these systems, as the actual contribution in only the fraction of N from legumes provided by biological N-fixation. Despite this limitation of this method, these coarse estimates still allow us to compare among systems. Harvested crop N exports included corn grain and silage (as corn was harvested for silage in S1 and S3 and grain in S2 and S4), soybean, and spelt. Exported N was calculated based on total kg/ha of biomass removed multiplied by an average %N content for each crop (corn silage 0.97% N, corn grain 1.27% N, soybeans 6.34% N, and spelt 2.29% N).

**Statistical analysis**

Analyses were completed in the software environment R (R Core Team 2018). To evaluate the effect of cropping system on total SIN over the growing seasons, we used a mixed-model repeated-measures approach with the statistical package lme4 (Bates et al. 2015). Total SIN was log-transformed, with cropping system, sampling date, and their interaction as fixed effects and block as a random effect. In this analysis, each start was analyzed separately to observe cumulative impacts of each system over time. An unstructured covariance matrix was used because of unequally spaced sampling through time. Post hoc pairwise comparisons of least squared means were performed with a Bonferroni correction for multiple pairwise tests ($\alpha = 0.05$). Corn population was also analyzed within each start separately, with cropping system as a fixed effect and block as a random effect (Champagne 2019).

For total aboveground biomass N and for deep soil SIN, we also used a mixed-model approach with log-transformed data. Cropping system was included as a fixed effect, with block and crop start included as random effects. In this case, we chose to include start as a random effect to target the effects of these four cropping systems across a range of environmental conditions and crop management legacies. Mean separations of significant effects were conducted using Tukey’s contrasts (glt) in the package multcomp (Hothorn et al. 2008).

**RESULTS AND DISCUSSION**

**Nitrogen inputs and exports**

In all systems within all starts, N inputs through manure and legume cover crops exceeded exports through crop harvest and removal (Table 2). This is an important consideration as agricultural systems with a surplus of N are susceptible to N losses to the environment. In all starts, S1 and S3 had a lesser N surplus than S2 and S4. This pattern was driven by differences in corn exports, with silage systems S1 and S3 having greater biomass N removed than grain systems S2 and S4. Silage systems had 200–370 kg N/ha exported through harvest, while grain systems had from 102 to 131 kg N/ha exported. As shown in Fig. 2, there was no significant difference in legume-derived N inputs among systems. Manure inputs included in these mass balance estimates represent the total N inputs from two manure applications, one before corn and one before spelt. An important N loss that we did not measure is ammonia; a significant percentage of N from liquid dairy manure may be lost as ammonia when it is left on the surface rather than immediately incorporated. This loss would have varied among systems based on manure incorporation method, with the injected manure in S4 presumably having lower ammonia losses compared with all other systems.

**Before corn crop planting**

In the fall preceding corn planting, S1 had manure applied, and both S1 and S2 had a tillage event, while S3 and S4 experienced little
disturbance during and after spelt harvest. The results of these management operations were as predicted, with S1 having the highest SIN availability through early fall with average peaks of 47 and 40 mg N/kg dry soil in September 2015 and 2016 compared with maximum average peaks of only 25 mg N/kg dry soil in S2, 8 mg N/kg dry soil in S3, and 10 mg N/kg dry soil in S4 (Fig. 3). There was 54% greater aboveground biomass N in the triticale in S1 (43 kg N/ha) than in S2 (28 kg N/ha; \( P = 0.0257 \)) and no significant difference in biomass N in the hairy vetch (S1 averaged 124 kg N/ha and S2 averaged 137 kg N/ha; Fig. 2). This difference in triticale N content was driven by greater triticale biomass, not by a difference in plant biomass C:N ratio, as the average C:N of triticale for both systems was 15.9. These data indicate that, depending on the magnitude of excess SIN in S1, N may have been taken up by the triticale instead of being lost to leaching.

The biggest N difference between the two legume + grass cover crop mixtures (hairy vetch + triticale vs. red clover + timothy) was the grass N content; timothy contained only 21–46% of the N in triticale (timothy averaged 13 kg N/ha in S3 and 9 kg N/ha in S4). A likely driver of this trend was the lower SIN availability in S3 and S4 compared with S1 and S2. By contrast, we found no differences in total aboveground legume biomass N among all four systems (Fig. 2). As predicted in all systems (Table 1), a mixture of a grass and legume cover crop had the dual function of scavenging SIN, reducing potential nitrate leaching, as well as supplying N through the legume biomass. These data suggest several considerations about N supply to corn from cover crops and manure. In systems with low SIN at the time of cover crop seeding, grass cover crops will not accumulate as much biomass, impacting the C:N ratio of the cover crop mixture. The ratio will become narrower, skewing toward the legume C:N ratio. Without a manure addition before cash

| Cropping system | Manure | Legume CC | Total inputs | Corn | Soybean | Spelt | Total exports | Balance |
|-----------------|--------|-----------|--------------|------|---------|-------|---------------|---------|
| 2015 Start S1   | 556    | 157       | 713          | 239  | 174     | 87    | 500           | 213     |
| S2              | 556    | 165       | 721          | 116  | 129     | 98    | 343           | 378     |
| S3              | 556    | 129       | 685          | 370  | 118     | 93    | 581           | 104     |
| S4              | 556    | 138       | 694          | 115  | 135     | 91    | 341           | 353     |
| 2016 Start S1   | 556    | 86        | 642          | 200  | 168     | 59    | 427           | 215     |
| S2              | 556    | 148       | 704          | 102  | 213     | 59    | 374           | 330     |
| S3              | 556    | 143       | 699          | 335  | 155     | 52    | 542           | 157     |
| S4              | 556    | 161       | 717          | 110  | 220     | 49    | 379           | 338     |
| 2017 Start S1   | 556    | 129       | 685          | 366  | 180     | 73    | 619           | 66      |
| S2              | 556    | 98        | 654          | 127  | 186     | 86    | 399           | 255     |
| S3              | 556    | 115       | 671          | 327  | 175     | 81    | 583           | 88      |
| S4              | 556    | 94        | 650          | 131  | 192     | 92    | 415           | 235     |

Notes: Mean N balance and standard error (in parentheses) for each system across years were calculated as 165 (40) for S1, 321 (29) for S2, 116 (17) for S3, and 309 (30) for S4. N inputs include two applications of liquid dairy manure and leguminous cover crops. N exports include three harvested cash crops (corn, soybean, and spelt). Corn was harvested for silage in system 1 (S1) and system 3 (S3), and for grain in system 2 (S2) and system 4 (S4). Balance was calculated by subtracting total export from total input. Start refers to the year that corn was planted within this corn–soybean–spelt rotation.
crop planting and without integrating this cover crop into the soil, SIN availability may be low and asynchronous with the cash crop demand. Furthermore, in systems with sufficient post-cover crop N addition inputs (in this case, through manure) impacts of the C:N ratio of the cover crop will not greatly impact the subsequent cash crop in terms of SIN availability.

**During the corn crop growing season**

Peak SIN in all systems followed the addition of N from either manure or aboveground cover crop biomass, as indicated in Fig. 3 by black downward-pointing arrows. Differences among systems in the magnitude of this SIN peak can be attributed to the preceding management practice. For example, in all starts, S1 (red) had a trend of

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Fig. 2. Average nitrogen content in aboveground cover crop biomass (kg N/ha) in four organic reduced-tillage cropping systems over three years. Crops with the same shaped icon were planted together in mixtures. Biomass samples were taken before cover crop termination either by a freeze (radish in the fall) or by mechanical termination (all other species in the spring). Each system incorporated one nitrogen-fixing legume designated by a green-filled triangle. Systems 2 and 4 incorporated a winter-killed species (radish) indicated by a magenta-filled circle. Cover crops were separated to species, except for Grass which represents the combination of annual ryegrass and orchardgrass, which were seeded together and are functionally similar. Error bars represent standard error.
Fig. 3. Soil inorganic nitrogen (mg N/kg dry soil) before and during corn crop growth in four organic reduced-tillage cropping systems. Systems differ in management of disturbance, cover crop timing and species, and manure application. Red, green, blue, and purple lines represent cropping systems treatments 1 through 4. Black vertical arrows show timing of key management operations (soil disturbance and/or manure applications, see systems diagram in Fig. 1 for detailed operations in each system). Yellow stripes at the top of each graph depict corn planting and growth. Triangles represent intermediate corn planting and harvest dates that differ by system. System 1 was planted later than all other systems, indicated by a downward-pointing red triangle. Silage harvest (systems 1 and 3) is indicated by the upward-pointing yellow triangle labeled Sil., while grain harvest (systems 2 and 4) is indicated by Gr., occurring at the end of the yellow stripe. Start refers to the year that corn was planted within this corn–soybean–spelt rotation. Error bars represent standard error.
lower SIN after corn planting because this system did not receive spring tillage or manure addition, while all other systems received manure coupled with spring tillage. In S1, average peak SIN in mg N/kg dry soil was 16, 21, and 18 in 2015, 2016, and 2017, respectively. All other systems saw average peaks above 30 and as high as 75 mg N/kg dry soil (Fig. 3). Instead of a spring manure application to supply the corn crop with N, S1 relied on mineralization of N from a decomposing cover crop. By not incorporating the cover crop, plant decomposition was delayed resulting in a slower release of N available to the corn. This supported our prediction that this component of S1—eliminating a tillage event by no-till planting a cash crop directly into terminated cover crop mulch—would result in low SIN that would not meet the N demands of the corn crop, as well as asynchrony between SIN and corn N demand (Table 1).

N availability in the summer during corn growth was similar among S2, S3, and S4, but SIN was significantly lower in S1 in the 2015 start and 2016 start (P < 0.001). Also, N export as silage yield was lower in S1 than in S3 by 35% in 2015 and 40% in 2016 (Table 2), which suggests that SIN availability in S1 was insufficient to meet crop demand. In contrast, in the 2017 start, SIN did not differ significantly among systems during corn growth. In fact, S1 had 12% higher N export as silage yield than S3. This may be attributed to differences in corn populations from early in the season; in 2017, S1 had greater corn populations than S3 (S1 had 68,000 plants/ha while S3 had 54,000 plants/ha; Table 3). Blind cultivation for weed management is a probable explanation for the population difference in this experimental year. Thus, while SIN availability is a crucial factor in yield output, other management and environmental variables were impacting corn yields as well. In other years, differences in weed biomass and cover crop biomass may have impacted corn establishment and subsequent N exports via yields.

At the time of cover crop interseeding in mid-July, SIN was significantly decreased in the 2015 start as it was drawn down to below 10 mg N/kg dry soil by the corn crop, but in the 2016 and 2017 starts, there was still SIN available for retention in the interseeded cover crops. Contrary to our prediction, we did not see direct evidence that a significant amount of N was being retained by the interseeded cover crops during the corn growing season. First, we saw no significant differences in SIN between interseeded treatments and non-interseeded treatments (Fig. 3). Second, we observed that the interseeded cover crops remained at a very low aboveground biomass from after establishment until corn senescence, most likely because of low light penetration through the corn canopy. This observation is supported by other work on interseeding, such as one study that measured the photosynthetic active radiation (PAR) beneath the corn canopy in an interseeded system and found that at corn reproductive tasseling stage, PAR was reduced by 80% at the soil surface (Brooker et al. 2020). During corn senescence and subsequent harvest, we observed the cover crops accumulating aboveground biomass at a higher rate. Because we sampled all cover crops at peak biomass in the late fall, our data capture total N accumulation during growth over the entire season but cannot be directly related to N retention during the period of highest SIN availability (July through September). In the late fall, average aboveground biomass N content of the interseeded cover crop was 11.4 kg N/ha in radish and 4.4 kg N/ha in grass

| Cropping system | Corn population 1000 plants/ha (SE) |
|-----------------|-----------------------------------|
| 2015 Start      |                                   |
| S1              | 73 (3.3) a                        |
| S2              | 77 (1.7) a                        |
| S3              | 74 (2.9) a                        |
| S4              | 77 (1.8) a                        |
| 2016 Start      |                                   |
| S1              | 41 (5.2) b                        |
| S2              | 65 (2.7) a                        |
| S3              | 60 (2.5) a                        |
| S4              | 62 (4.5) a                        |
| 2017 Start      |                                   |
| S1              | 68 (4.2) a                        |
| S2              | 65 (1.9) a                        |
| S3              | 54 (5.8) b                        |
| S4              | 66 (3.1) a                        |

Note: SE, standard error. Systems (S1–S4) differ in management of disturbance, cover crop timing and species, and manure application. Letters a and b indicate Tukey’s honestly significant difference at P = 0.05 within each Start year. Start refers to the year that corn was planted within this corn–soybean–spelt rotation.
(combined annual ryegrass + orchardgrass). In the post-harvest cover crop planting, average cereal rye biomass N content was 4.7 kg N/ha. While these total aboveground N contents were different between interseeded and non-interseeded systems, they did not drive any differences in SIN in the top 20 cm of soil (Fig. 3). It is possible that there were differences in SIN in deeper soil layers that we were not able to measure, especially considering the known ability of radish to scavenge and retain deep soil N (Wang and Weil 2018).

After the corn crop growing season

Deep soil cores collected in spring 2018 following the corn and its subsequent cover crop revealed differences in the distribution of SIN throughout the soil profile (Fig. 4). There was a significant difference in SIN among systems in the 40–60 cm depth ($P = 0.0173$) and the 60–80 cm depth ($P = 0.0096$). In particular, interseeded corn grain systems (S2 and S4, interseeded with radish + annual ryegrass + orchardgrass) had higher SIN in deeper soil layers, which may have been susceptible to loss through soil water leaching. While the levels of SIN were not high in these deep soil layers (all levels were below 5 mg N/kg dry soil), this pattern is important to investigate as it may persist in systems with higher N inputs resulting in higher SIN levels, potentially contributing significant amounts of N to a leachable pool. We attribute this pattern to several interacting factors. First, the interseeded mixture contained proportionally less established grass than the monoculture cereal rye in the spring. Radish has broad leaves and a large fleshy root and is not a winter-hardy species. Interseeded systems with a high population of radish in the fall could result in a sparsely covered field in the spring after radish die-off (Wallace et al. 2020). Additionally, these systems receive a pulse of N from the decomposition of the winter-killed radish. This N, if not retained by a robust grass in early spring, may be susceptible to spring leaching (Dean and Weil 2009). Another factor contributing to this SIN pattern at depth is that the cereal rye cover crop (S1 and S3) may have accumulated greater spring biomass N than the ryegrass + orchardgrass cover crop mixture (S2 and S4) due to belowground growth differences dictated by inherent species traits. For example, a study in Maryland found cereal rye...
to have a higher percent recovery rate of April SIN than annual ryegrass because of its rapid growth in cool weather (Shipley et al. 1992). In our experiment, the cereal rye may have been able to grow faster and deeper than the ryegrass + orchardgrass mixture in the spring, allowing it to access deeper N pools. This may have contributed to the difference in total aboveground cover crop N between the interseeded grass mixture and cereal rye (Fig. 2). Greater spring scavenging and retention by the post-corn harvest planted cereal rye coupled with the deep soil SIN data indicate that a quick-growing, high-biomass monoculture grass cover crop is better for N retention than an interseeded mixture of a winter-killed brassica and lower-biomass grasses.

It is important to note that the ecological complexity combined with the lack of controlled comparisons in this systems experiment makes the trade-offs between these cover cropping strategies a challenge to interpret. Other work has shown N retention benefits from interseeding cover crops when compared with a non-cover cropped control (Noland et al. 2018, Alonso-Ayuso et al. 2020). This supports the idea that environmental conditions, SIN status, soil type, and associated risk of cover crop establishment are all key considerations when designing a cover cropping scheme for N retention. Further targeted research is needed to parse out mechanisms of N retention and loss resulting from using interseeded cover crop mixtures in order to fully understand the impact of this strategy on N management.

Emergent patterns

The importance of prioritizing N retention in cover crops was clearly demonstrated in this experiment, since all cropping systems had greater N inputs than exports through crop harvest at the end of the three-year rotation. Cover crop species traits, timing of manure application, and timing of tillage contributed to differences in total aboveground biomass N. Although cover crop mixtures can provide multiple services (Finney and Kaye 2017), they may not always be the strongest choice to target the goal of N retention. In this experiment, a fast-growing high-biomass grass planted after corn harvest (S1 and S3) outperformed a mixture of a lower-biomass grain and a winter-killed brassica (S2 and S4). In corn grain systems, interseeding this specific cover crop mixture may not provide maximum N retention benefits. However, the strategy of resource partitioning by cover crops could provide N benefits in other systems where there is more time for unhindered fall cover crop growth—such as a corn silage system—or if different species of interseeded cover crops are selected to better target N retention. Cover cropping outcomes will depend on environmental conditions, SIN status, and associated risk of cover crop establishment. As shown in this work, cover crop species selection, phenology, and functional group are important considerations when integrating mixtures or monocultures into a complex cropping system.

Timing of tillage and manure application played a major role in SIN availability and subsequent retention in both cash and cover crops. While tillage intensity (via moldboard plow or chisel plow) did not seem to directly impact the N balance over the three-year rotation, tillage timing and frequency and manure application timing did impact temporal dynamics of SIN availability. For example, during the corn phase of the rotation, S1 was designed to reduce tillage by no-till planting corn into a rolled-down cover crop mulch, relying on the decomposition of that cover crop to supply N. As predicted, this stood out as an N-asynchronous system in which N was over-supplied to the cover crop planted before corn and under-supplied for the demands of the corn crop. This system, when compared to the other silage system S3, had greater N surplus in 2 out of 3 years, indicating that this system may not provide as much N retention as silage system S3. In S3, termination of the cover crop mixture before corn by tillage allowed for earlier corn planting, and therefore earlier silage harvest and earlier cereal rye establishment after corn—all components that functioned to increase N retention in this system compared with S1.

Conclusions

Managing N in ecologically based agroecosystems has inherent challenges with implications for both farmers and the environment. Integrating reduced-tillage and cover cropping strategies simultaneously leads to a complexity of interactions with both intended and unintended results.
This experiment aimed to evaluate four cropping systems that integrate these ecological strategies in different combinations and at different time points in a three-year organic grain crop rotation. Using a systems approach, this experiment lays a foundation for farmers and researchers in designing ecologically based agroecosystems to achieve low N pollution outputs.

Because all four of the experimental systems incorporated manure N inputs, N-fixing cover crops, high-biomass cover crops, and reduced-tillage strategies, it was possible that they all would have performed similarly. On the overall system level, N retention and supply outcomes could not be readily predicted from the sum of the parts. Instead, interactions among system components were important drivers of temporal N dynamics. Neither interseeding cover crop mixtures nor reducing tillage resulted in marked N benefits—we did not observe improved N retention from either practice in this case, nor were they associated with an increase in N uptake by corn. What clearly emerged from this experiment is the importance of managing for synchrony between SIN availability and cash crop N demand as influenced by the N retention capacity of cover crops and the timing of N mineralization due to tillage. In systems such as these that require high N inputs, it is crucial to manage for this synchrony to promote retention and reduce N losses to the environment while maintaining crop yields and economic sustainability.

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Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3380/full