Nitrogen Concentration and Nitrogen Mineralization by Nonleguminous Cover Crops: Applications to Central Oregon Cropping Systems

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# Abstract

Nonleguminous cover crops established in late summer and early fall have become increasingly popular in Central Oregon. Growers in the region are interested in working with mustard (*Brassica juncea*), tillage radish (*Raphanus sativus* L. var. *oleiformis*), and oat as cover crops. These cover crops have the ability to recycle N to a subsequent spring-planted cash crop after overwintering and tillage incorporation in the spring. Soil N levels required to achieve maximum N concentration in the plant tissue and maximum N mineralization potential are not known for this region. The purpose of this incubation experiment was to observe the effect of N fertilizer rate on cover crop N concentration and to observe the effect of cover crop N concentration on N mineralization. Cover crops were grown in the greenhouse in 0.2 m2 plastic seeding flats with granular urea-N fertilizer rates of 0, 45, 90 and 135 kg N ha-1. After 10 weeks of growth, plants were harvested and a subsample was dried, ground and analyzed for total N concentration.

Subsamples of fresh cover crop species were mixed with Madras loam soil and aerobically incubated in polyethylene bags at 3.4, 3.7 and 2.3 g cover crop residue per kg soil on a dry weight basis for mustard, oat and radish, respectively. Soil in incubation bags was subsampled at 4 and 8 weeks to determine nitrate-N concentration. Cover crop N percentage on a dry weight basis increased proportionally with increasing N fertilizer rates for all three cover crop species in the study. Specifically, mustard tissue N concentrations were 2.5, 3.3, 3.7 and 3.8% N at 0, 45, 90 and 135 kg N ha-1 fertilizer rates, respectively, oat tissue N concentrations were 2.4, 3.0, 3.7 and 4.2% over the same respective N fertilizer rates, and radish tissue N concentrations for the same fertilizer rates were 2.2, 3.1, 3.4 and 3.9%, respectively. At 8 weeks, the amount of mineralized nitrate-N (NO3-N) for mustard, oat and radish were 61, 107 and 69 mg kg-1 at the 90 kg N ha-1 rate and 80, 106 and 76 mg kg-1 at the 135 kg N ha-1 rate. Nitrogen rates of 90 and 135

kg N ha-1 produced cover crops with the highest N concentrations and highest levels of N mineralization compared to the lower rates of 0 and 45 kg N ha-1. Nitrogen rate influenced N concentration in cover crop residues more than plant species for these early harvested cover crops.

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# Introduction

Cover crops have multiple benefits: they improve soil structure, mitigate erosion, stimulate soil microbial activity, reduce soil pathogen load and accumulate nitrogen (N). A catch crop is a cover crop grown for its deep root system that is used to recover soil N. A portion of this N is recycled to a subsequent cash crop when the cover crop is tilled into the soil and the N mineralizes from the decomposing residues. Nonleguminous cover crops accumulate residual soil N left from a previous cash crop by virtue of their deep and extensive root systems. A deep root system is the fundamental characteristic of a catch crop and the essence of its N harvesting capability (Kristensen and Thorup-Kristensen, 2004).

Late-summer-planted small grain and brassica (mustard and tillage radish) cover crops have become popular in the Central Oregon region, which includes Crook, Deschutes and Jefferson counties. This region is the largest production area for hybrid carrot seed in the United States. Central Oregon used to be a major irrigated soft white spring wheat production zone.

Wheat acreage has diminished to 6% of cultivated land in Jefferson county as of 2018 (Gill,2018). Irrigated wheat is no longer an economically high-value crop in Central Oregon but it still serves an important function as a rotational crop. The reason wheat is a rotational crop is that it does not serve as a pest reservoir for alfalfa and carrots.

Central Oregon is an irrigated agricultural production zone. In years that water allotments are 1.5-2.0 acre feet (feet of water that could accumulate on the surface of an acre of land), there is sufficient water to grow both a spring cash crop and a subsequent cover crop from germination until late-fall dormancy. Cover crops provide several agroecosystem services, from erosion control to residual soil nitrogen (N) recovery remaining from a previous cash crop. Growers

want to know how much N is needed to establish a nonleguminous overwintering cover crop, how much N that cover crop will return to a subsequent cash crop and when that N will be released from tillage-incorporated cover crop residues.

Cover crops seeded in late-summer or early-fall following a spring cash crop serve several agroecosystem services. They improve soil structure by increasing aggregation around their root systems which improves water infiltration and retention (Dabney et al., 2001). Feeder roots are constantly dying and being replaced and this contributes more organic matter to the soil. Organic matter enriches the soil microbial community that mediates the cycling of soil organic nitrogen to plant-available mineralized N (Dabney et al., 2010).

Winter cover crops protect the soil from wind and water erosion. Ground covering vegetation over winter is of crucial importance where precipitation primarily falls in winter and spring in Central Oregon and winds can be high. Without a cover crop that thoroughly covers the ground, soil is prone to erosion losses. Brassica crops, like mustards and radishes, can serve as an effective ground covers when planted at an appropriate time for fast growth. Brassica are capable of producing 8,967 kg/ha dry matter biomass (Chen et al., 2007). Timing is crucial in planting brassica covers. If they are planted in cold climates after the first week in September they are at risk of not producing a sufficient amount of biomass to prevent soil erosion losses. This was shown in 2002 mustard planting date trial at Moses Lake, WA (McGuire, 2018).

The inclusion of small grains having deep fibrous root systems and brassicas having deep fleshy root systems can increase soil aggregation and improve water infiltration (Dabney et al., 2001; McGuire, 2003). The reason for increased soil particle aggregation is that the constantly decomposing feeder roots provide a food source for soil microbes whose populations increase greatly. These microbes secrete various extracellular exudates that adhere soil particles to each

other, creating a stronger aggregate structure. The more aggregates there are, the greater the amount of macropores in the soil for increased gravitational water flow down the soil profile during a rain event. An additional way in which a heavy stand of cover crop aids in water infiltration and decreased soil erosion is that vegetative cover deflects rain drops and slows their velocity (Dabney et al., 2001).

The greatest advantage afforded by cover crops when there is residual N remaining in a field after a spring cash crop is by using the root systems of these cover crops to accumulate this

1. Nitrogen accumulated in the aboveground biomass and root systems of the cover crop is brought closer to the soil surface from deeper soil layers. When these residues are tillage- incorporated, organic N compounds in the plant tissue is mineralized to the plant available form of nitrate-N (Kristensen and Thorup-Kristensen, 2004; Thorup-Kristensen, 2006). If subsequent crop planting is timed to coincide with N mineralization and cash crop N requirements, this can result in savings of fertilizer N. The cover crops that would be used in this scenario are typically nonleguminous, since the purpose is not to use a legume to fix N but rather a deep-rooted small grain or brassica to intercept residual N. The purpose of these catch crops is to keep as much N as possible in the system for future cash crop use and that as little N as possible is lost by leaching from the root zone. Central Oregon has shallow soils typically around 60 cm deep, underlain by fractured basalt. In these soils N loss through leaching with winter rain is likely if there isn’t a root system that will intercept it.

Brassicas have two types of root systems: Those of mustards and rapeseed are branching while radishes have deep taproots. Both root systems are thick, possessing considerable biomass and can reach depths of 2 m (Chen et al., 2007). H.L. Kristensen and K. Thorup-Kristensen have studied N-scavenging from deep soil layers in multiyear crop rotation studies and in 15N tracer

studies. In a study comparing the rooting depths and N uptake of various catch crops and subsequent cash crops, forage radish (*Raphanus sativus* L. var. *oleiformis*) had observed rooting depths of 2.5 m in 30% of samples (Thorup-Kristensen, 2006).

The value of a brassica cover crop for removing N from deeper soil layers and returning it to the surface, making it available to a shallower-rooted cash crop, is clearly seen in the same study (Thorup-Kristensen, 2006). At 2 m, in a fallow plot before which radish had been planted, inorganic N concentration was at 10 kg ha-1 and at 0.25 m was 85 kg ha-1. When leeks were planted as a cash crop following bare soil, they had a N concentration of 112 kg ha-1 yet when following forage radish, they had a statistically significant N concentration increase, N uptake being 151 kg ha-1.

Knowing the ideal N application rate to establish a healthy stand of a small grain or brassica cover crop is important. This is necessary because only a ground-covering stand will be capable of accumulating large amounts of soil N and also serve an erosion control and biofumigation role. Equally important is knowing the N synchrony of the N mineralized from decomposing cover crop residues in relation to the N requirements of the subsequent cash crop. Synchrony is the temporal coordination N mineralization with the phenological stage at which a crop accumulates N. Synchrony is the most important consideration when using cover crops to recycle N. Understanding N synchrony in a cover crop-cash crop pairing is the key to reducing N fertilizer inputs and preventing N loss from the system. Gaining the knowledge of N mineralization over time is the reason for conducting residue incubations. Once researchers know the timing of peak N mineralization from cover crop residues, planting dates can be decided for subsequent cash crops to maximize N synchrony.

There are few studies that consider the effect of different N rates in the soil on cover crop N mineralization to a subsequent crop. Specific N rates needed to establish an overwintering nonleguminous catch crop for the N recycling purpose are little-studied. Research studies have been focused on N retrieval by catch crops at the soil N level was already present in the soil (Kristensen and Thorup-Kristensen, 2004) or at a non-limiting N fertilizer rate was used to establish the cover crop (Vos and Van der Putten, 1997). Studies focused on the amount of N that is needed to grow a ground covering stand of a brassica or small grain that will accumulate and recycle an economically viable amount of N in excess of what is needed to establish the crop are limited. Angus et al. (Angus et al., 1991) evaluated wheat yield as a function of cover crop species by N-rate. The study included three N rates: 0, 40, and 80 kg N ha-1. Brown mustard resulted in a statistically significant higher wheat yield at both the 40 and 80 kg N ha-1 fertilizer rates. At the 80 kg N ha-1 rate, it continued to produce statistically-significantly higher wheat yields while all of the other cover crop and N-fertilizer combinations at that rate reached a yield plateau. This study cannot be used to make predictions about plant tissue N concentration in nonleguminous cover crops as a function of soil N level because the research plot had been previously planted to clover. Even when N was not added, there was sufficient N in the field and that the soil not limited in N supply. Thus, a baseline level of soil N fertility necessary to induce N-mineralization rather than N-immobilization in a nonleguminous cover crop cannot be inferred from this study.

All additional studies that considered N mineralization from nonleguminous cover crops have added single nonlimiting N rates. Collins et al., Trinsoutrot et al., and Vos and van der Putten (Trinsoutrot et al., 2000a; Vos and Van der Putten, 2001; Collins et al., 2007) all conducted laboratory incubations to quantify N release over time. In the absence of multiple N

rates, mineralization data from these studies is of limited value because it does not provide information on N mineralization dynamics at multiple levels of soil N. Research is needed on optimal soil N levels and fertilizer N rates to establish a nonleguminous N catch crop in Central Oregon.

Growers are already using overwintering brassica and small grain cover crops but they lack guidance regarding the amount of N needed to maximize these crops’ N recycling abilities to a subsequent spring/summer cash crop. Field studies in which brassica and small grain cover crops are grown at different soil N rates are needed to establish an N budget that weighs N inputs against N losses. A graphic example of the N budget concept can be seen in figure 1.



Figure 1. Nitrogen recycling timeline from late-summer cover crop planting to subsequent cash crop planting

It is advantageous to link N rate field studies in cover crops to aerobic laboratory incubations of field-grown cover crop residues to determine optimal N mineralization potential of the cover crops. Together, these linked experiments can be used to determine both optimal soil N or fertilizer N to grow nonleguminous N catch crops in Central Oregon and also how to time

their tillage-incorporation to achieve N synchrony with a subsequent cash crop in the spring or summer. By including multiple N rates as an experimental treatment, N mineralization data and N uptake synchrony with a subsequent cash crop can be found that can be used to determine a spectrum of pre-cover crop planting soil N rates from deficient to optimal for N mineralization of the incorporated residues.

The ability of a catch crop to return N to a subsequent cash crop is of economic importance to growers because the amount of N recycled can be deducted from fertilization rates. Weinert et al. ( 2002) took 180 cm soil samples at spring incorporation of separately- planted cover crops, including small grains and mustard, through the harvest of a subsequent potato crop. They also took aboveground cover crop biomass N samples and obtained N concentrations of potato plants at 3-4 week intervals 40-60 day intervals from planting until harvest. This intensive sampling allowed the authors to trace the N return from the cover crop to the cash crop relatively closely. They found that cover crops increased N accumulation in comparison to bare fallow control through tuber initiation at 47 days after planting. The difference between cover crop plot N accumulation and bare fallow control was 99.6 to 56.4 kg N ha-1. These findings are useful for estimating N available to the succeeding crop starting at preplant, but do not indicate the amount of N available to crops over the course over the growing season.

Isotopic tracer studies are useful because they allow for the partitioning of N pools which facilitates the calculation of N replacement values from added N. In the absence of tracer N, separating the contribution of added N from soil organic N mineralization or any residual N from a prior crop becomes more challenging. Another study in the Columbia Basin traced isotopic 15N from a mustard cover crop to a subsequent potato cash crop (Weinert et al., 2002). The

researchers harvested the aboveground biomass of the cover crop which recovered a maximum 51% of the 56 kg 15N ha-1 applied. 29% of total N taken up by the cover crop was recycled to the following potato crop, which resulted in a maximum N replacement value of 40 kg N ha-1 (Collins et al., 2007). Vos and van der Putten (2001) estimated that the average fertilizer replacement value of rye, rapeseed, and radish cover crops to a subsequent potato cash crop to be 61%. They did not quantify the individual cover crops’ fertilizer replacement values, however.

Producers are interested in new cultural techniques that result in yield and quality increases that return profits beyond the cost of adopting novel agronomic and pest management strategies. For this reason, it is important to evaluate the effect of nonleguminous cover crops on cash crop yield and quality. A three-year cover crop to cash crop study in Austria evaluated cover crop mixtures consisting of legumes, non-legumes, and a legume non-legume mix for their N accumulation and re-supply value when planted in midsummer and incorporated in late fall as green manure. The non-legume cover crop mixture, which included forage radish and turnip (*B. rapa* var. *rapa*) resulted in the same yield for barley (*Hordeum vulgare*) compared to bare fallow (Rinnofner et al., 2008).

A nonleguminous cover crop can be more of a liability than an asset if it does not have a sufficiently high N concentration to be a source of mineralizable N. An 8-year winter cover crop study following various summer vegetable crops was carried out in Salinas, California from 2003-2011. The winter cover crops were not supplied their own source of N and were solely taking up residual N from vegetable cash crops which had different N requirements. Overall, it was found that the mustards used in the cover crop treatments had N concentrations ranging from 37-41 g kg-1 N. This concentration is suitable for N mineralization, the threshold for net N mineralization from cover crop dry matter being 14-18 g kg-1 N (Brennan et al., 2013).

There are several studies that treat cover crop N mineralization as a function of carbon:nitrogen (C:N) ratio and biochemical composition of crop residues (Quemada and Cabrera, 1995; Wagger et al., 1998; Trinsoutrot et al., 2000a; b), namely describing the difference in mineralization rates between different parts of the plant as a function of chemical groups in them. In an incubation study, at the high rate of N applied, oilseed radish residues had less cellulose and lignin than at the low rate of applied N, which resulted in faster N mineralization (Trinsoutrot et al., 2000b). In addition to these incubation studies, there are equations to estimate N mineralized from crop residues which include C:N ratio and N concentration in the equations. Using data from six experiments in the literature and two that they conducted, Vigil and Kissel determined from an equation they developed that the C:N ratio separating net mineralization and net immobilization is 40 (Vigil and Kissel, 1991).

The studies that are the subject of this report seek to test the following three hypotheses:

* 1. Nitrogen concentration in young mustard, radish, and oat cover crop residues is a function of N fertilization rate.
	2. Nitrogen mineralization is a function of N concentration in young mustard, radish, and oat cover crop residues.
	3. Nitrogen mineralized at room temperature will be highest at 4 weeks of incubation and by the 8th week, there will be little N release from young mustard, radish, and oat cover crop residues.

Two experiments were conducted to investigate the N mineralization characteristics of late- summer planted and spring incorporated nonleguminous cover crops in an irrigated production system in Central Oregon. The first experiment was a field study and the second experiment was a laboratory investigation. In the field experiment, three cover crop treatments, brown mustard,

forage oats, and oilseed radish were established with four rates of urea-N fertilizer (0, 45, 90, 135 kg N ha-1) in the late summer, tillage incorporated the subsequent spring and then a crop of soft white spring wheat was grown. The second experiment was a laboratory incubation of cover crop residues, with soil samples for N mineralization taken at 4 and 8 weeks, respectively. The objective of the field study was to observe the effect of different N rates and cover crop types on yield and protein content of wheat at harvest. The objectives of the laboratory incubation experiment were: to observe the effect of N rate on cover crop N concentration and to observe the effect of N concentration on N mineralization. Cover crops failed to establish in the field and had to be regrown in the greenhouse. Because of this, the following paper will only discuss the results of the laboratory incubation in relation to the stated objectives for that experiment. Wheat harvest will not be discussed.

# Materials and Methods

The first experiment was a field study in which the three overwintering cover crops: brown mustard (*Brassica juncea* “Caliente”), forage oat (*Avena sativa* “Charisma”) and tillage radish (*Raphanus sativus* L. var. *oleiformis*) were to be grown in the field at four rates of urea-N to simulate either residual N left after a previous cash crop or to determine an ideal N rate to establish these cover crops for highest ground cover and optimal N concentration for acceptable nitrate-N (NO3-N) mineralization to a subsequent late-spring/summer cash crop (see figure 1 in introduction for a general schematic of the crop rotation). The cover crops were planted on 7 September 2017. Later on, it was determined that the planting date may have been too late to insure a healthy stand of cover crops over the winter. If brassicas are planted in cold climates after the first week of September in locations such as the Columbia basin in Washington (Central

Oregon being an analogous climate), they will not produce significant biomass, as was shown in a 2002 mustard planting date trial at Moses Lake, WA (McGuire et al., 2018).

# Field Selection

The field experiment was conducted at the Central Oregon Agriculture Research and Extension Center (COAREC) in Madras, Oregon. The soil series in the research plot, according to its location on the map in the NRCS soil survey of Jefferson County, Oregon is Madras loam (Fine- loamy, mixed, superactive, mesic Aridic Argixerolls). The research plot was 1.05 acres (0.4 hectares). The field had been previously planted to a winter wheat variety trial that was harvested on 18 August 2017. The wheat was cut to a height of 15 cm and this stubble remained in the field. Initial ammonium-N (NH4-N) concentrations in the field at 15 cm and 30 cm were 0.506 and 0.374 mg kg-1 NH4-N, respectively. For NO3-N at the 15 and 30 cm depths, the respective NO3-N concentrations were 1.18 and 0.451 mg kg-1.

# Cover Crop Planting

Cover crops were planted and N fertilizer (granular urea—46% N) was applied on 7 September 2017. The experiment was a 4 x 4 factorial arranged in a randomized complete block design. The three cover crop treatments, planted in individual subplots, were: brown mustard (*Brassica juncea* “Caliente 199”), tillage radish (*Raphanus sativus* L. var. oleiformis), and forage oats (*Avena sativa* “Charisma”). The fourth cropping condition treatment was a fallow treatment.

Each treatment had one of four rates of granular urea applied: 0 kg ha-1, 45 kg ha-1, 90 kg ha-1, or 135 kg ha-1. The cover crop or bare ground x fertilizer treatments combined to twelve cover crop and four bare ground treatments, for a total of 16 separate treatments. Each combination of 16 treatments was replicated four times, for a total of 64 plots, each plot measuring 9 m long by 5 m

wide. The 16 treatments were in north to south oriented vertical rows, with a 1 m gap between plots. The repetitions were separated horizontally east-to-west, with 2.5 m of separation between them.

**2017 Winter Cover Crop-Spring Wheat PAN Trial**

30ft^2 subplot length+ 4 ft b/w plot x 16 plots/rep --> 34' x 16 = 544ft^2 length N-->S each rep

|  |  |  |  |
| --- | --- | --- | --- |
| REP 1 | REP 2 | REP 3 | REP 4 |
| 6 | 5 | 13 | 11 |
| 13 | 4 | 16 | 3 |
| 16 | 13 | 9 | 6 |
| 15 | 1 | 7 | 1 |
| 10 | 11 | 3 | 16 |
| 14 | 6 | 1 | 13 |
| 3 | 2 | 11 | 7 |
| 4 | 8 | 14 | 4 |
| 2 | 3 | 2 | 8 |
| 1 | 9 | 10 | 5 |
| 12 | 15 | 4 | 12 |
| 5 | 16 | 8 | 14 |
| 9 | 12 | 6 | 2 |
| 8 | 7 | 5 | 15 |
| 11 | 10 | 12 | 9 |
| 7 | 14 | 15 | 10 |

|  |
| --- |
| Treatment |
| 1 | Mustard-- 0 kg/ha N |
| 2 | Mustard-- 45 kg/ha N |
| 3 | Mustard-- 90 kg/ha N |
| 4 | Mustard-- 135 kg/ha N |
| 5 | Radish-- 0 kg/ha N |
| 6 | Radish-- 45 kg/ha N |
| 7 | Radish-- 90 kg/ha N |
| 8 | Radish-- 135 kg/ha N |
| 9 | Oats-- 0 kg/ha N |
| 10 | Oats-- 45 kg/ha N |
| 11 | Oats-- 90 kg/ha N |
| 12 | Oats-- 135 kg/ha N |
| 13 | Bare soil-- 0 kg/ha N |
| 14 | Bare soil-- 45 kg/ha N |
| 15 | Bare soil-- 90 kg/ha N |
| 16 | Bare soil-- 135 kg/ha N |

544ft

16ft^2 subplot width + 5ft^2 tractor turn radius = 21ft^2 rep width x 4 reps= 84ft^2 E-->W width of plot

|  |
| --- |
| Amount Seed Needed |
| Mustard: 5,760ft^2 @ 12 lbs/ac | 1.59 lbs mustard seed |
| Radish: 5,760ft^2 @ 8 lbs/ac | 1.06 lbs radish seed |
| Oats: 5,760ft^2 @ 90 lbs/ac | 11.9 lbs oat seed |

84ft

Seed requirement: 480ft^2/plot x 3 treatments x 4 replications = 5,760ft^2 coverage for each cover crop

Planted area: 30'x16'= 480'x12 treatmentsx 4 repetitions=

480'x48'= 23,040 ft^2= 0.53 ac

Total plot size: 544ft^2 length x 84ft^2 width= 45,696ft^2= 1.05ac

Figure 2. Field plot diagram

The field was disked and harrowed to break up the mat of wheat stubble from the prior trial and to establish a firm seedbed for planting. A Great Plains 1200 Mounted Min-Till Drill (Great Plains Mfg. Salina, KS) was used to plant the cover crops. The seeding rates were 13.5 kg ha-1, 9 kg ha-1, and 101 kg ha-1 for mustard, radish, and oats, respectively. Mustard seeding rate was based on an OSU extension publication (Wysocki, 2002) and that for forage radish was from a USDA plant materials technical note (Hybner, 2014). Seeding rate for the oats was in accordance with guidance in *Using Cover Crops in Oregon* (Sattell and Dick, 1998). Mustard

and radish planting depths were both 0.6 cm and oats were planted at a depth of 1.9 cm using a seed drill. The seeds were covered with soil by the packing wheels on the seed drill. Spacing between seed rows within each subplot was 17.8 cm.

Because of the small size of the plots, the fertilizer box on the drill could not be effectively used to apply fertilizer. Therefore, fertilizer application was accomplished by hand broadcasting, distributing an approximately equal amount of granular urea first along the vertical north-to-south line of the plots, and then along the horizontal east-to-west line. Volunteer wheat from the previous crop and other weeds were allowed to germinate prior to cover crop germination by means of one immediate irrigation set of 4 cm water to sprout weeds. Germinated weeds were sprayed once with glyphosate (Monsanto, Saint Louis, MO) on 12 September 2017 as a measure to prevent weeds from outcompeting the cover crop. In addition to the initial irrigation, the cover crop was provided with 10 cm of irrigation water in addition to that used for weed germination. Cover crops were planted later than intended because a prior wheat crop in the experimental plot could not be harvested until late August. When mustard and radish are planted after the first week of September at high altitude locations in the inland Pacific Northwest, germination is often poor.

Insufficient heat units were accumulated before winter dormancy for the mustard and radish to either establish a ground-covering stand or to grow more than approximately 3 cm tall. The forage oats did establish in sporadic dense patches in their plots, growing to approximately 15 cm. Because of the poor cover crop growth at the tillage incorporation date of 26 March 2018, the field study could not be continued with the intention of observing NO3-N mineralization from tillage incorporated residues and its recycling to a subsequent wheat crop. Cover crops were re- grown in the greenhouse to generate the required biomass for the laboratory incubation, the

second experiment. Soil from the field plots was still collected on this date for the incubation bags and wheat was planted on 28 March 2018.

# Preplant Soil N Sampling

Preplant soil samples were collected on 26 March 2018. Five soil samples were taken to a 15 cm depth along a diagonal southwest to northeast transect in each subplot. The samples were collected with 2 cm diameter open-sided soil push probes and were thoroughly mixed to create a composite sample for each subplot. These samples were then air dried in the greenhouse at the COAREC for 72 hours and ground with a mortar and pestle to pass a 2 mm sieve. The air-dried and ground samples were then brought to the Central Analytical Laboratory (CAL) at OSU for KCl extraction and colorimetric analysis per the method in *Soil, Plant and Water Reference Methods for the Western Region* (Gavlak et al., 2003). The method is as follows: 7.5 g soil sample from each subplot was mixed in a 50 mL centrifuge tube (VWR, Radnor, PA) with 30 mL 2 M KCl for 1 hr at low speed in a reciprocating shaker. The resulting supernatant was then filtered through Whatman #1 filter papers (Whatman PLC, Maidstone Kent, UK). This solution was then analyzed through colorimetric methods; NO3-N was reduced using a cadmium reduction column and NH4-N was heated with salicylate and hypochlorite in an alkaline phosphate buffer. Both processes produce colorimetric reactions that could then be quantitatively analyzed for NO3-N and NH4-N in proportion to the color opacity using a Lachat 8500 Series 2 Flow Injection Analysis System (Lachat Instruments, Loveland, CO).

Soil NO3-N content was quantified for each cover crop x fertilizer rate treatment at the beginning of the 8-week laboratory incubation. It was decided that soil from the in-field subplots that had corresponding greenhouse cover crop x fertilizer rate seedling trays would serve this purpose. The mean of the soil NO3-N of the 4 replications of each in-field treatment, including

the soil-only treatments, was taken as the week 0, or initial soil NO3-N, for the purpose of the laboratory incubation. This decision was made to preserve some form of continuity between the field experiment and the laboratory incubation.

Additionally, a 1 gallon (3.8 L) zipper-top plastic storage bag of soil from each subplot was collected and held in a walk-in cooler (Imperial Manufacturing, Portland, OR) at 4oC until use in the cover crop incubation study. Soil was collected from a single point in each subplot with a shovel to a 30 cm depth. The objective was to collect 1 kg of soil so there would be an ample amount for the incubation study. There was a substantial amount of wheat stubble from the previous wheat crop that was removed from the field before cover crop planting in addition to rocks. Wheat stubble has a high C:N ratio and the possibility of N immobilization from this residue, which was not removed from the soil bags existed.

# Greenhouse Cover Crop Planting

Cover crop samples had to be grown in the greenhouse at the COAREC in order to obtain sufficient biomass for the lab incubation study to determine N mineralization rate from cover crop residues. This was necessary because the cover crops did not produce substantial biomass in the field and could not be harvested *in situ*. The greenhouse grown cover crops were sown in 0.6 m long by 0.3 m wide by 0.2 m deep seedling flats with seeding and fertilizer rates adjusted to an area of 0.18 m2, the surface area of the flats. Seeding rates were as follows: mustard—13.5 kg

ha-1—0.3 g/tray, radish—9 kg ha-1—0.2 g/tray, oats—101 kg ha-1—2.1 g/tray and fertilizer rates

were: 45 kg N ha-1 —2.1 g urea/tray, 90 kg N ha-1—4.2 g urea/tray, 135 kg N ha-1—6.3 g urea/tray. The seeding trays did not have holes in the bottom, minimizing potential N leaching. There were three cover crop treatments by four fertilizer rate treatments by four replications for a

total of 48 trays. There was no randomization; trays were grouped by replication in ascending order of fertilizer rate, by cover crop.

Rep 1

Rep 2

Rep 3

Rep 4

Mustard 0 kg/ ha N

Mustard 0 kg/ ha N

Mustard 0 kg/ ha N

Mustard 0 kg/ ha N

Mustard 45 kg/ ha N

Mustard 45 kg/ ha N

Mustard 45 kg/ ha N

Mustard 45 kg/ ha N

Mustard 90 kg/ ha N

Mustard 90 kg/ ha N

Mustard 90 kg/ ha N

Mustard 90 kg/ ha N

Mustard 135 kg/ ha N

Mustard 135 kg/ ha N

Mustard 135 kg/ ha N

Mustard 135 kg/ ha

Oat 0 kg/ ha N

Oat 0 kg/ ha N

Oat 0 kg/ ha N

Oat 0 kg/ ha N

Oat 45 kg/ ha N

Oat 45 kg/ ha N

Oat 45 kg/ ha N

Oat 45 kg/ ha N

Oat 90 kg/ ha N

Oat 90 kg/ ha N

Oat 90 kg/ ha N

Oat 90 kg/ ha N

Oat 135 kg/ ha N

Oat 135 kg/ ha N

Oat 135 kg/ ha N

Oat 135 kg/ ha N

Radish 0 kg/ ha N

Radish 0 kg/ ha N

Radish 0 kg/ ha N

Radish 0 kg/ ha N

Radish 45 kg/ ha N

Radish 45 kg/ ha N

Radish 45 kg/ ha N

Radish 45 kg/ ha N

Radish 90 kg/ ha N

Radish 90 kg/ ha N

Radish 90 kg/ ha N

Radish 90 kg/ ha N

Radish 135 kg/ ha N

Radish 135 kg/ ha N

Radish 135 kg/ ha N

Radish 135 kg/ ha N

N

Figure 3. Greenhouse cover crop growth diagram: seeding trays were 0.18 m2

Soil for the trays was collected from the margins of the experimental field with a shovel to a 30 cm depth on 26 March 2018. Fertilizer was hand incorporated into each tray at the specified rate for each treatment and then cover crop seeds were sown on top of the soil to ensure as even a distribution across the surface of the tray as possible and were gently tamped into the

soil to guarantee good seed-soil contact for optimum germination. Trays were initially watered to saturation and thereafter as it appeared necessary from looking at and touching the soil surface.

The greenhouse cover crop plants were sown on 29 March 2018. By 11 June 2018 sufficient biomass had accumulated to allow for the cover crop samples to be uprooted, washed of soil, and frozen at 0oC until use in the lab cover crop incubation study, beginning on 27 June 2018. The cover crops were all in the vegetative growth stage and approximately 10 cm tall at the time of collection. Only above-soil plant matter was retained for use in the incubation experiment.

# Cover Crop Residue Tissue Analysis for Total N

A single sample of each cover crop species by N rate treatment was oven dried at 60oC in a VWR Oven F Air 2.3 CF oven (VWR International, Radnor, PA) for 72 hours to determine the percent moisture content for the amount of fresh residue to add for each cover crop type on a dry weight basis. Fresh weights of the mustard, oat and radish samples were 4.1, 10 and 3.3 g, respectively. Weights after 72 hr oven drying for mustard, oat and radish were 0.6, 1.5 and 0.3 g, respectively. Additionally, a 2.0 g sample of each cover crop treatment was oven dried in the same manner and then ground in a Cyclotec 1093 sample mill (Foss A/S, Hillerod, Denmark) so that a 150 mg sample thereof could later be analyzed for total N content in the CAL’s Elementar Vario MACRO Cube (Elementar Americas, Ronkonkoma, NY) in accordance with the protocol in *Soil, Plant and Water Reference Methods for the Western Region* (Gavlak et al., 2003) for dry combustion to determine total N.

# Laboratory Cover Crop Incubation Study

Stored soil samples were removed from the walk-in cooler on 27 June 2018 and allowed to equilibrate with room temperature. Frozen cover crop samples were taken out of the freezer and cut into 15 mm pieces. Approximately 650 g of field soil, at the moisture content it was

collected, which was assumed to be near field capacity (20% volumetric water content moisture for a loam), and corresponding to the treatment and repetition subplots of the cover crop residues was hand mixed in 3.8 L Ziploc (S.C. Johnson & Sons, Racine, WI) plastic freezer bags with approximately 6.5 g cover crop residue, dry weight basis. Weights were not exact because soil samples were not sieved to separate residual wheat straw from the prior field trial and rocks. The average soil bag weight for the field moist soil was 680 g and these values ranged from a high of 773 g to a low of 653 g with a standard deviation of 19. The bags were sealed with the exception of a plastic drinking straw inserted in the corner of each bag to allow for aerobic respiration.

Ratios of dry cover crop tissue biomass weight to dry soil weight were approximated as follows for mustard, oat and radish, respectively: 1:294, 1:276 and 1:435. These ratios were 3.4 g mustard residue kg-1 soil, 3.7 g oat residue kg-1 soil and 2.3 g radish residue kg-1 soil. The soil weights for each incubation bag by cover crop type and fertilizer N treatment were highly variable.

Table 1: Incubation bag weight information showing variability in bag weights of field moist soil and estimation of dry soil weight based on estimated volumetric water content (VWC)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Cover Crop | N fertilizer rate | Incubation bag soil fresh weight means (actual) | Range in soil fresh weights (n= 4) | Incubation bag soil dry weight estimated means\* | Range in estimated soil dry weights\* |
|  | kg/ha | g | g | g | g |
| Mustard | 0 | 673.2 | 13 | 538.5 | 10.4 |
|  | 45 | 673.9 | 13.7 | 539.1 | 11.0 |
|  | 90 | 691.4 | 44.2 | 553.1 | 35.4 |
|  | 135 | 709.8 | 107.6 | 567.8 | 86.1 |
| Oat | 0 | 681.8 | 21.8 | 545.4 | 17.4 |
|  | 45 | 682.1 | 32.7 | 545.7 | 26.2 |
|  | 90 | 675.2 | 7.1 | 540.2 | 5.7 |
|  | 135 | 676.1 | 21.5 | 540.9 | 17.2 |
| Radish | 0 | 689.8 | 47.8 | 551.8 | 38.2 |
|  | 45 | 680.9 | 13.4 | 544.7 | 10.7 |
|  | 90 | 675.7 | 12.9 | 540.6 | 10.3 |
|  | 135 | 686.6 | 27.5 | 549.3 | 22.0 |

\*VWC estimated at 20%

For decomposition to proceed, soil needed to be kept moist. This was done by misting the soil in each bag with water on a weekly basis. No volumetric water content was established and water was added to the point that the surface soil in each bag was moistened to the point of color change and the appearance of a glistening sheen. Then, the surface soil was incorporated with the rest of the soil in the bag by massaging the outside of the bag to mix all the soil homogeneously.

The incubation experiment took place over eight weeks, with soil being removed at 28 and 56 days, respectively. This soil was then air dried for 72 hours in the COARC greenhouse

and ground to pass a 2 mm sieve. After this it was stored prior to KCl extraction and quantitative colorimetric analysis at CAL to determine NO3-N content, as described above.

# Results

Cover crop N concentration increased with increasing N fertilizer rate. The N concentration increase was best represented by a quadratic equation for the three cover crops. The quadratic equations for the trendlines of mustard, oat and radish were: -0.00000005x2 + 0.0195x + 2.5465,

-0.000005x2 + 0.0143x + 2.407 and -0.00004x2 + 0.0173x + 2.2735, respectively. The R2 values

for mustard, oat and radish were 0.99, 0.99 and 0.98, respectively.



Figure 4: Mean tissue N concentration as function of N fertilizer rate after 10 weeks of growth in a greenhouse setting for aboveground biomass. Plants were grown in a Madras loam soil. Four replications in a non-randomized design.

Cover crop N concentrations were relatively homogeneous within fertilizer rate treatments across cover crop species.

Table 2. Mean tissue N concentration as function of N fertilizer rate after x weeks of growth in a greenhouse setting for aboveground biomass. Plants were grown in a Madras loam soil. Four replications in a non-randomized design.

|  |  |  |  |
| --- | --- | --- | --- |
| N fertilizer rate in greenhouse media | Mustard | Oat | Radish |
| kg/ha | %(n= 4) | %(n= 4) | %(n= 4) |
| 0 | 2.54 | 2.41 | 2.24 |
| 45 | 3.29 | 3.03 | 3.07 |
| 90 | 3.67 | 3.66 | 3.40 |
| 135 | 3.81 | 4.24 | 3.90 |

Average percent moisture content was assumed from a single oven dried sample of each cover crop species. The lack of fresh-to-dry weight ratios for all cover crop samples precludes verification of the hypothesis that lower moisture content in the oat residues at the 135 kg N ha-1 fertilizer rate caused a higher % N relative to the other cover crops. The single samples yielded 14, 15 and 8% dry matter for mustard, oat and radish, respectively.

Nitrogen concentrations in terms of N added to soil incubation bags as a function of N fertilizer rate added to greenhouse trays (table 3) shows the cover crop N concentration data more explicitly than the %N data in table 1. The markedly lower dry matter content in the radish sample points to a possible dilution of N that could explain the lower %N in comparison to mustard and oat. At the 45 kg N ha-1 fertilizer rate, there is only a 4 mg kg-1 difference in added N between mustard and oat. Between mustard and radish, the difference in added N is 53 mg kg-1 at the same fertilizer rate.

Table 3. Nitrogen added with cover crop dry matter to incubation bags. Soil used was a Madras loam.

|  |
| --- |
| Total N added via cover crop biomass |
| N fertilizer rate in greenhouse media | Mustard (n= 4) | Oat (n= 4) | Radish (n= 4) |
| kg/ha | mg/kg | mg/kg | mg/kg |
| 0 | 81 | 89 | 49 |
| 45 | 119 | 115 | 66 |
| 90 | 129 | 139 | 80 |
| 135 | 125 | 161 | 89 |

The effect of cover crop species on N mineralization relies on a difference equation of cover crop incubation soil minus a soil-only control. The difference between the soil N concentration of a soil sample from an incubation treatment with a particular cover crop species residue at a certain fertilizer level treatment is subtracted a soil-only N concentration in which the soil N concentration is a known, uniform value. The N mineralization rate is determined by dividing the difference of these two values by the time between each sampling period, which in the case of this experiment was 4 and 8 weeks.

Mean soil NO3-N mineralization over the 4 week and 8 week incubation time steps (figure 5) was relatively uniform across fertilizer treatments. The NO3-N mineralization for the 45 kg N ha-1 fertilizer level at week 4 was 25 mg NO3-N kg-1 soil and at the 135 kg N ha-1 treatment it was 32 mg NO3-N kg-1 soil. The increased mineralization at week 8 for the two

levels was 12 and 10 additional mg NO3-N kg-1 soil. There was still a substantial amount of N to be mineralized at 8 weeks. The wide standard error (SE) of the mean bars at the highest fertilizer treatment in this figure are important to note. They both indicate high variability and overlap with the SE bars of the other treatments, which had statistics been performed in this experiment, would have been a sign of lack of statistical difference between fertilizer treatments on NO3-N mineralization. The high variability in standard error of the treatment means made it difficult to detect a treatment effect.

Week 4

Week 8

Figure 5. NO3-N concentration in soil-only incubation bags at 4 and 8 weeks. Error bars are standard error of mean (n=4). Madras loam soil.

Cover crop residue NO3-N mineralization is presented for each cover crop at the 4 and 8- week incubation time steps in figures 6-8. There is a trend of increased soil NO3-N as a factor of increased cover crop residue N concentration for the mustard and radish cover crop residues (figures 6-8). This trend occurs at both time steps, though in radish at the highest residue N concentrations, there is less of a mineralization increase as there is for mustard. At the 3.7 and

3.8% N concentration levels in mustard, soil NO3-N was 52 and 66 mg NO3-N kg-1 soil at 4- weeks and 61 and 80 mg NO3-N kg-1 soil at 8-weeks. In radish at the 4 and 8-week time steps for residue N concentrations of 3.4 and 3.9% N, soil NO3-N levels were 60 and 72 and 69 and 76 mg NO3-N kg-1 soil, respectively. For oat, the mineralization differences at the highest N concentration differences, 3.7 and 4.2 % N are negligible. What is prominent about the data from oat is the higher soil NO3-N levels in comparison to mustard and radish. The soil NO3-N recovery at week 4 at the 135 kg N ha-1 fertilizer rate for mustard, oat, and radish was 66, 100 and 72 mg NO3-N kg-1 soil, respectively. The mean NO3-N of the soil samples for mustard and radish differ by 6 mg kg-1 yet the gap between mustard and oat and radish and oat is 34 and 28 mg kg-1, respectively.

Week 4

Week 8

Figure 6. Mustard NO3-N concentration in incubation bags at the 4 and 8 week sampling time steps. Cover crop N concentrations are averages from dry combustion analysis (see table 1). Error bars are standard error of the mean (n=4).

Week 4

Week 8

Figure 7. Oat NO3-N concentration in incubation bags at the 4 and 8 week sampling time steps. Cover crop N concentrations are averages from dry combustion analysis (see table 1). Error bars are standard error of the mean (n=4)

Week 4

Week 8

Figure 8. Radish NO3-N concentration in incubation bags at the 4 and 8 week sampling time steps. Cover crop N concentrations are averages from dry combustion analysis (see table 1). Error bars are standard error of the mean (n=4).

Nitrogen mineralization did not increase between weeks 4 and 8 for oat and radish crop at any residue N concentration level. At the highest residue N concentration levels for oat and radish, the difference between week-4 and week-8 soil NO3-N recovery was 6 and 4 mg NO3-N kg-1 soil, respectively. Mustard had a 12 mg kg-1 soil NO3-N increase at the highest residue N concentration between the two time steps.

# Discussion

The results of the incubation experiment showed releases of NO3-N in quantities ranging from 46 to 245 kg ha-1 from all cover crop residues over the entire 8-week period, which is enough N to reduce N fertilizer application rates for the succeeding crop. In comparison to values in table 3 of “Predicting plant-available nitrogen with the OSU Organic Fertilizer and Cover Crop Calculator (Sullivan et al., not yet published), cover crops with N concentrations of 2%, at the low end of the range of concentrations of residues from the incubation experiment, produce 4 lb N dry ton-1 residue in 4 weeks and 10 lb N dry ton-1 in 10 weeks. At a 4% N concentration, at the high end of cover crop residue N concentration in the incubation experiment, 36 lb N dry ton-1 is expected at 4 weeks and 40 lb N dry ton-1 at 10 weeks. Therefore, to achieve ideal N return from a cover crop, it can either be fertilized with a 90 or 135 kg N ha-1 rate at establishment, the rates in this experiment that resulted in residue concentrations of approximately 4% or follow a crop that leaves large amounts of residual N, such as potato.

Cover crop termination at an appropriate growth stage is crucial for two reasons: first, if a cover crop has not produced sufficient biomass, the amount of N to be recouped will be minimal and second, if a cover crop is terminated too late and a subsequent crop planted soon thereafter,

the N from the cover crop might not be available to meet cash crop demands. An additional consideration in termination timing concerns residue quality. This is an issue with a high C:N cover crop such as forage oat, as was used in this incubation experiment. As a small grain crop matures, its C:N ratio steadily increases to the point that it generally is a poor N mineralizer or a short-term N immobilizer. This is not a concern for the most part with fall-planted brassica cover crops such as the brown mustard and tillage radish used in the experiment, as their N concentrations are similar to legumes, containing 37-41 g N kg-1 shoot dry weight (Brennan and Boyd, 2012).

If forage oat is to be terminated before jointing, in order to avoid a significant decrease in N concentration (and a subsequent increase in C:N ratio) that would render it a potential N immobilizer, it will produce only about 1 ton dry matter, analogous to what has been found in N utilization literature from the Pacific Northwest (Sullivan et al., 1999) with wheat serving as the example for a small grain. In the same publication, the aggregation of data from multiple field studies in the Willamette Valley found that maximum wheat accumulation of N was 120 lb ac-1. From this example, it would appear that the maximum amount of mineralized N to be furnished from a small grain cover crop at a growth stage at which it would still be a net N mineralizer is 12 lb ac-1. This is a negligible amount and not worth fertilizing if it will be planted as a cover crop. Therefore, before any small grain is to be used as a cover crop with the goal of N recycling to a subsequent cash crop, it would be advisable to conduct a soil N test to determine if the amount in the field is sufficient to produce a ground-covering small grain cover crop without need for additional input.

The cover crops used in this incubation experiment were late-summer/ early-fall-planted cover crops that can be incorporated into the soil before potential winter kill in Central Oregon,

as would likely happen for the tillage radish, with less of a risk for more cold hardy brown mustard; forage oat is cold tolerant and will survive cold winters. Cover crops can be overwintered and then incorporated in early spring so that N recovered by the cover crops can be furnished to the subsequent late-spring/ early-summer cash crop. The risk of N loss is reduced both if all cover crops survive the winter and even if winter kill does occur in the dry and cold conditions of Central Oregon, decomposition of dead mustard and radish tissue is not the significant risk in this region that it is in the Mid-Atlantic (Dean and Weil, 2009).

The experimental design of this incubation was flawed in two key ways. First, soil-only controls used in determining N mineralization were not taken from a location with a uniform soil N level across the sampling area. Second, measurement of all experimental units was imprecise. If this experiment is to be repeated, the control N samples for mineralization calculations should be taken from a field in which no crop has been grown and no N fertilizer added so that N levels in the field are at an ambient “natural” level. Quadrat sampling should potentially be employed with multiple KCl extractions run per quadrat to verify that N levels across the plot used for the control soil has an even N distribution.

The incubation protocol was also imprecise in all measures relating to proper ratios of cover crop residue to soil. Straw from the previous wheat crop and rocks were in field soil bags that detracted from the overall weight of soil in each bag. Additionally, there was no known gravimetric water content for the soils, making conversion to volumetric water content at field capacity an impossibility. This reduced the weekly addition of water to the bags to a simple matter of replacing water in the on a gravimetric basis to the point that the bags weighed the same each week as they did at the initiation of the experiment, the assumption being that weight loss was primarily due to evaporative water loss without consideration of CO2 evolution in the

course of microbial respiration contributing to weight reduction in the soil bags. In the future, all cover crop samples should have a portion oven-dried to accurately determine fresh-weight additions on a dry-weight basis. All soil samples for the incubation bags should similarly have a subsample oven-dried to determine gravimetric water content for conversion to volumetric water content to be used in computing the amount of water to add on a volumetric basis to achieve field capacity for the soil texture in question. Soils should also be wet-sieved to separate all debris and particles above 2 mm in diameter. Wet-sieving is essential because a rewetted air or oven-dried soil will not have the same biological capability as a field moist soil.

# Conclusions

There were some important trends to support the hypotheses tested: Increasing N fertilizer rates increased N concentration in cover crop residues. The 90 and 135 kg N ha-1 fertilizer rates produced cover crops with the highest N concentrations and the highest levels of N mineralization. In contrast to the hypothesis that mineralization would be mostly complete at 4 weeks, equal levels of N mineralization were seen at 8 weeks. A two-month mineralization window extends the availability of cover crop-derived N to a subsequent cash crop, increasing the ability to achieve synchrony and meet crop N demands over a wider time period and even provide some N to a winter crop.

It is clear from the substantially lower N concentrations in the cover crop residues from the 0 kg N ha-1 treatment and the much lower mineralized N amounts that a large amount of N must either remain in the soil following a cash crop or be added to the nonleguminous overwintering cover crop to obtain a substantial amount of mineralized N for a subsequent cash crop.

The field experiment was critical to the framework of this research: quantifying the N inputs and losses followed by N content in the grain of the subsequent wheat crop would have allowed the generation of a preliminary N budget for overwintering nonleguminous catch crops in Central Oregon. Additionally, the residues from the field-grown catch crops were to be used in the laboratory incubation with soil from the same in-field treatment subplots. This would have provided mineralization data more indicative of field conditions than having had to grow the cover crops in the greenhouse and then incubate them in field soil from the respective treatment subplots. If conclusive inferences are to be drawn concerning the use of nonleguminous overwintering mustard and radish N catch crops in Central Oregon, a successful field experiment must be carried out. Experimental methods for both the field and laboratory experiments must be improved to reduce experimental error and allow the statistical analysis of defensible results.

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# Appendix

# Appendix:

The following appendix contains the raw data for the incubation experiment

Table 4. Plot-by-plot data of cover crop N percentage, cover crop N added to incubation bags and soil NO3-N concentration over 8 week incubation period.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Cover Crop** | **Week of incubation** | **Fertilizer rate** | **Total N in Cover Crop** | **Cover Crop N added in incubation** | **Soil NO3-N concentration (mg N/ kg soil)** |
|  |  |  | kg/ha | % | mg N/incubation bag | mg/kg |
| 102 | Bare soil | 0 | 0 |  |  | 11 |
| 203 | Bare soil | 0 | 0 |  |  | 8 |
| 301 | Bare soil | 0 | 0 |  |  | 6 |
| 406 | Bare soil | 0 | 0 |  |  | 7 |
| 106 | Bare soil | 0 | 45 |  |  | 16 |
| 216 | Bare soil | 0 | 45 |  |  | 3 |
| 308 | Bare soil | 0 | 45 |  |  | 14 |
| 412 | Bare soil | 0 | 45 |  |  | 11 |
| 104 | Bare soil | 0 | 90 |  |  | 34 |
| 211 | Bare soil | 0 | 90 |  |  | 12 |
| 316 | Bare soil | 0 | 90 |  |  | 18 |
| 414 | Bare soil | 0 | 90 |  |  | 19 |
| 103 | Bare soil | 0 | 135 |  |  | 28 |
| 212 | Bare soil | 0 | 135 |  |  | 26 |
| 302 | Bare soil | 0 | 135 |  |  | 44 |
| 405 | Bare soil | 0 | 135 |  |  | 38 |
| 102 | Bare soil | 4 | 0 |  |  | 26 |
| 203 | Bare soil | 4 | 0 |  |  | 20 |
| 301 | Bare soil | 4 | 0 |  |  | 21 |
| 406 | Bare soil | 4 | 0 |  |  | 13 |
| 106 | Bare soil | 4 | 45 |  |  | 37 |
| 216 | Bare soil | 4 | 45 |  |  | 23 |
| 308 | Bare soil | 4 | 45 |  |  | 25 |
| 412 | Bare soil | 4 | 45 |  |  | 14 |
| 104 | Bare soil | 4 | 90 |  |  | 21 |
| 211 | Bare soil | 4 | 90 |  |  | 22 |
| 316 | Bare soil | 4 | 90 |  |  | 22 |
| 414 | Bare soil | 4 | 90 |  |  | 30 |
| 103 | Bare soil | 4 | 135 |  |  | 16 |
| 212 | Bare soil | 4 | 135 |  |  | 59 |
| 302 | Bare soil | 4 | 135 |  |  | 22 |
| 405 | Bare soil | 4 | 135 |  |  | 29 |

II

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Cover Crop** | **Week of incubation** | **Fertilizer rate** | **Total N in Cover Crop** | **Cover Crop N added in incubation** | **Soil NO3-N concentration (mg N/ kg soil)** |
|  |  |  | kg/ha | % | mg N/incubation bag | mg/kg |
| 102 | Bare soil | 8 | 0 |  |  | 41 |
| 203 | Bare soil | 8 | 0 |  |  | 31 |
| 301 | Bare soil | 8 | 0 |  |  | 42 |
| 406 | Bare soil | 8 | 0 |  |  | 25 |
| 106 | Bare soil | 8 | 45 |  |  | 48 |
| 216 | Bare soil | 8 | 45 |  |  | 36 |
| 308 | Bare soil | 8 | 45 |  |  | 39 |
| 412 | Bare soil | 8 | 45 |  |  | 24 |
| 104 | Bare soil | 8 | 90 |  |  | 30 |
| 211 | Bare soil | 8 | 90 |  |  | 31 |
| 316 | Bare soil | 8 | 90 |  |  | 32 |
| 414 | Bare soil | 8 | 90 |  |  | 45 |
| 103 | Bare soil | 8 | 135 |  |  | 30 |
| 212 | Bare soil | 8 | 135 |  |  | 67 |
| 302 | Bare soil | 8 | 135 |  |  | 29 |
| 405 | Bare soil | 8 | 135 |  |  | 33 |
| 110 | Mustard | 0 | 0 | 3.01 | 110 | 2 |
| 204 | Mustard | 0 | 0 | 2.07 | 69 | 2 |
| 306 | Mustard | 0 | 0 | 2.05 | 79 | 4 |
| 404 | Mustard | 0 | 0 | 3.02 | 67 | 1 |
| 109 | Mustard | 0 | 45 | 2.92 | 106 | 3 |
| 207 | Mustard | 0 | 45 | 3.09 | 118 | 2 |
| 309 | Mustard | 0 | 45 | 3.25 | 115 | 2 |
| 413 | Mustard | 0 | 45 | 3.92 | 138 | 2 |
| 107 | Mustard | 0 | 90 | 4.25 | 140 | 13 |
| 209 | Mustard | 0 | 90 | 2.81 | 94 | 2 |
| 305 | Mustard | 0 | 90 | 3.51 | 139 | 9 |
| 402 | Mustard | 0 | 90 | 4.13 | 143 | 0 |
| 108 | Mustard | 0 | 135 | 3.61 | 109 | 14 |
| 202 | Mustard | 0 | 135 | 3.22 | 102 | 9 |
| 311 | Mustard | 0 | 135 | 4.30 | 143 | 22 |
| 408 | Mustard | 0 | 135 | 4.12 | 143 | 17 |
| 110 | Mustard | 4 | 0 | 3.01 | 110 | 3 |
| 204 | Mustard | 4 | 0 | 2.07 | 69 | 19 |
| 306 | Mustard | 4 | 0 | 2.05 | 79 | 14 |
| 404 | Mustard | 4 | 0 | 3.02 | 67 | 8 |
| 109 | Mustard | 4 | 45 | 2.92 | 106 | 8 |
| 207 | Mustard | 4 | 45 | 3.09 | 118 | 38 |
| 309 | Mustard | 4 | 45 | 3.25 | 115 | 38 |

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| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Cover Crop** | **Week of incubation** | **Fertilizer rate** | **Total N in Cover Crop** | **Cover Crop N added in incubation** | **Soil NO3-N concentration (mg N/ kg soil)** |
|  |  |  | kg/ha | % | mg N/incubation bag | mg/kg |
| 413 | Mustard | 4 | 45 | 3.92 | 138 | 40 |
| 107 | Mustard | 4 | 90 | 4.25 | 140 | 84 |
| 209 | Mustard | 4 | 90 | 2.81 | 94 | 40 |
| 305 | Mustard | 4 | 90 | 3.51 | 139 | 60 |
| 402 | Mustard | 4 | 90 | 4.13 | 143 | 25 |
| 108 | Mustard | 4 | 135 | 3.61 | 109 | 45 |
| 202 | Mustard | 4 | 135 | 3.22 | 102 | 50 |
| 311 | Mustard | 4 | 135 | 4.30 | 143 | 78 |
| 408 | Mustard | 4 | 135 | 4.12 | 143 | 91 |
| 110 | Mustard | 8 | 0 | 3.01 | 110 | 1 |
| 204 | Mustard | 8 | 0 | 2.07 | 69 | 39 |
| 306 | Mustard | 8 | 0 | 2.05 | 79 | 25 |
| 404 | Mustard | 8 | 0 | 3.02 | 67 | 27 |
| 109 | Mustard | 8 | 45 | 2.92 | 106 | 2 |
| 207 | Mustard | 8 | 45 | 3.09 | 118 | 62 |
| 309 | Mustard | 8 | 45 | 3.25 | 115 | 58 |
| 413 | Mustard | 8 | 45 | 3.92 | 138 | 69 |
| 107 | Mustard | 8 | 90 | 4.25 | 140 | 103 |
| 209 | Mustard | 8 | 90 | 2.81 | 94 | 50 |
| 305 | Mustard | 8 | 90 | 3.51 | 139 | 88 |
| 402 | Mustard | 8 | 90 | 4.13 | 143 | 3 |
| 108 | Mustard | 8 | 135 | 3.61 | 109 | 51 |
| 202 | Mustard | 8 | 135 | 3.22 | 102 | 76 |
| 311 | Mustard | 8 | 135 | 4.30 | 143 | 87 |
| 408 | Mustard | 8 | 135 | 4.12 | 143 | 106 |
| 113 | Oats | 0 | 0 | 3.07 | 106 | 1 |
| 210 | Oats | 0 | 0 | 1.88 | 72 | 0 |
| 303 | Oats | 0 | 0 | 2.70 | 106 | 1 |
| 415 | Oats | 0 | 0 | 1.97 | 71 | 1 |
| 105 | Oats | 0 | 45 | 3.19 | 113 | 1 |
| 215 | Oats | 0 | 45 | 3.34 | 133 | 0 |
| 310 | Oats | 0 | 45 | 2.80 | 111 | 1 |
| 416 | Oats | 0 | 45 | 2.78 | 103 | 1 |
| 115 | Oats | 0 | 90 | 3.25 | 132 | 0 |
| 205 | Oats | 0 | 90 | missing | missing | 2 |
| 307 | Oats | 0 | 90 | 3.37 | 127 | 0 |
| 401 | Oats | 0 | 90 | 4.38 | 160 | 1 |
| 111 | Oats | 0 | 135 | 4.87 | 208 | 4 |
| 213 | Oats | 0 | 135 | 3.33 | 121 | 0 |

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| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Cover Crop** | **Week of incubation** | **Fertilizer rate** | **Total N in Cover Crop** | **Cover Crop N added in incubation** | **Soil NO3-N concentration (mg N/ kg soil)** |
|  |  |  | kg/ha | % | mg N/incubation bag | mg/kg |
| 315 | Oats | 0 | 135 | 4.22 | 145 | 0 |
| 411 | Oats | 0 | 135 | 4.55 | 168 | 3 |
| 113 | Oats | 4 | 0 | 3.07 | 106 | 0 |
| 210 | Oats | 4 | 0 | 1.88 | 72 | 3 |
| 303 | Oats | 4 | 0 | 2.70 | 106 | 6 |
| 415 | Oats | 4 | 0 | 1.97 | 71 | 0 |
| 105 | Oats | 4 | 45 | 3.19 | 113 | 55 |
| 215 | Oats | 4 | 45 | 3.34 | 133 | 48 |
| 310 | Oats | 4 | 45 | 2.80 | 111 | 43 |
| 416 | Oats | 4 | 45 | 2.78 | 103 | missing |
| 115 | Oats | 4 | 90 | 3.25 | 132 | 66 |
| 205 | Oats | 4 | 90 | missing | missing | 122 |
| 307 | Oats | 4 | 90 | 3.37 | 127 | 112 |
| 401 | Oats | 4 | 90 | 4.38 | 160 | 92 |
| 111 | Oats | 4 | 135 | 4.87 | 208 | 122 |
| 213 | Oats | 4 | 135 | 3.33 | 121 | 92 |
| 315 | Oats | 4 | 135 | 4.22 | 145 | 106 |
| 411 | Oats | 4 | 135 | 4.55 | 168 | 82 |
| 113 | Oats | 8 | 0 | 3.07 | 106 | 1 |
| 210 | Oats | 8 | 0 | 1.88 | 72 | 17 |
| 303 | Oats | 8 | 0 | 2.70 | 106 | 2 |
| 415 | Oats | 8 | 0 | 1.97 | 71 | 1 |
| 105 | Oats | 8 | 45 | 3.19 | 113 | 60 |
| 215 | Oats | 8 | 45 | 3.34 | 133 | 36 |
| 310 | Oats | 8 | 45 | 2.80 | 111 | 57 |
| 416 | Oats | 8 | 45 | 2.78 | 103 | 1 |
| 115 | Oats | 8 | 90 | 3.25 | 132 | 64 |
| 205 | Oats | 8 | 90 | missing | missing | 126 |
| 307 | Oats | 8 | 90 | 3.37 | 127 | 126 |
| 401 | Oats | 8 | 90 | 4.38 | 160 | 112 |
| 111 | Oats | 8 | 135 | 4.87 | 208 | 114 |
| 213 | Oats | 8 | 135 | 3.33 | 121 | 88 |
| 315 | Oats | 8 | 135 | 4.22 | 145 | 126 |
| 411 | Oats | 8 | 135 | 4.55 | 168 | 96 |
| 112 | Radish | 0 | 0 | 1.74 | 39 | 1 |
| 201 | Radish | 0 | 0 | 2.47 | 56 | 3 |
| 314 | Radish | 0 | 0 | 2.21 | 49 | 1 |
| 410 | Radish | 0 | 0 | 2.55 | 51 | 4 |
| 101 | Radish | 0 | 45 | 3.79 | 74 | 5 |

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| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Cover Crop** | **Week of incubation** | **Fertilizer rate** | **Total N in Cover Crop** | **Cover Crop N added in incubation** | **Soil NO3-N concentration (mg N/ kg soil)** |
|  |  |  | kg/ha | % | mg N/incubation bag | mg/kg |
| 206 | Radish | 0 | 45 | 2.42 | 59 | 4 |
| 313 | Radish | 0 | 45 | 2.68 | 57 | 1 |
| 403 | Radish | 0 | 45 | 3.41 | 73 | 1 |
| 116 | Radish | 0 | 90 | 2.87 | 63 | 1 |
| 214 | Radish | 0 | 90 | 3.78 | 101 | 1 |
| 304 | Radish | 0 | 90 | 2.42 | 62 | 6 |
| 407 | Radish | 0 | 90 | 4.55 | 95 | 7 |
| 114 | Radish | 0 | 135 | 3.15 | 77 | 2 |
| 208 | Radish | 0 | 135 | 4.09 | 106 | 4 |
| 312 | Radish | 0 | 135 | 4.50 | 92 | 25 |
| 409 | Radish | 0 | 135 | 3.85 | 81 | 10 |
| 112 | Radish | 4 | 0 | 1.74 | 39 | 6 |
| 201 | Radish | 4 | 0 | 2.47 | 56 | 17 |
| 314 | Radish | 4 | 0 | 2.21 | 49 | 1 |
| 410 | Radish | 4 | 0 | 2.55 | 51 | 10 |
| 101 | Radish | 4 | 45 | 3.79 | 74 | 46 |
| 206 | Radish | 4 | 45 | 2.42 | 59 | 46 |
| 313 | Radish | 4 | 45 | 2.68 | 57 | 1 |
| 403 | Radish | 4 | 45 | 3.41 | 73 | 23 |
| 116 | Radish | 4 | 90 | 2.87 | 63 | 46 |
| 214 | Radish | 4 | 90 | 3.78 | 101 | 52 |
| 304 | Radish | 4 | 90 | 2.42 | 62 | 40 |
| 407 | Radish | 4 | 90 | 4.55 | 95 | 102 |
| 114 | Radish | 4 | 135 | 3.15 | 77 | 37 |
| 208 | Radish | 4 | 135 | 4.09 | 106 | 56 |
| 312 | Radish | 4 | 135 | 4.50 | 92 | 82 |
| 409 | Radish | 4 | 135 | 3.85 | 81 | 112 |
| 112 | Radish | 8 | 0 | 1.74 | 39 | 0 |
| 201 | Radish | 8 | 0 | 2.47 | 56 | 34 |
| 314 | Radish | 8 | 0 | 2.21 | 49 | 1 |
| 410 | Radish | 8 | 0 | 2.55 | 51 | 2 |
| 101 | Radish | 8 | 45 | 3.79 | 74 | 53 |
| 206 | Radish | 8 | 45 | 2.42 | 59 | 68 |
| 313 | Radish | 8 | 45 | 2.68 | 57 | 2 |
| 403 | Radish | 8 | 45 | 3.41 | 73 | 33 |
| 116 | Radish | 8 | 90 | 2.87 | 63 | 55 |
| 214 | Radish | 8 | 90 | 3.78 | 101 | 50 |
| 304 | Radish | 8 | 90 | 2.42 | 62 | 55 |
| 407 | Radish | 8 | 90 | 4.55 | 95 | 116 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Cover Crop** | **Week of incubation** | **Fertilizer rate** | **Total N in Cover Crop** | **Cover Crop N added in incubation** | **Soil NO3-N concentration (mg N/ kg soil)** |
|  |  |  | kg/ha | % | mg N/incubation bag | mg/kg |
| 114 | Radish | 8 | 135 | 3.15 | 77 | 23 |
| 208 | Radish | 8 | 135 | 4.09 | 106 | 62 |
| 312 | Radish | 8 | 135 | 4.50 | 92 | 96 |
| 409 | Radish | 8 | 135 | 3.85 | 81 | 122 |