

# Measuring active carbon to predict seasonal nitrate mineralization on organic farms

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## Project Summary

Determining how much fertilizer to apply is a delicate process; too much fertilizer is a potential waste of money and can lead to nutrient loss while too little fertilizer will not optimize yield. Efficient fertilization requires giving appropriate credit for the nitrogen (N) mineralization potential of a soil, which can be significant in historically amended soils. Predicting N mineralization is problematic because there is often poor correlation between net N mineralization and information in typical soil tests, such as percent soil organic matter. Fast, simple, and cheap methods to predict N mineralization are desirable for farmers, especially those that rely heavily on soil building practices such as cover cropping and manure for soil fertility.

In this study, N mineralization from plots with no applied fertilizer was estimated at 8 certified organic farms in western Washington State with two separate *in situ* methods: plant uptake and buried soil tubes. Uptake of mineral N by broccoli transplants over an average of 76 days ranged from 42 to 131 kg N ha<sup>-1</sup> (37 to 117 lb N acre<sup>-1</sup>) and mineralization in buried soil tubes over 182-day period (2,674 growing degree days, GDD) ranged from 25 to 53 mg NO<sub>3</sub>-N + NH<sub>4</sub>-N kg soil<sup>-1</sup> (91 to 156 kg ha<sup>-1</sup> or 81 to 139 lb N acre<sup>-1</sup>). The two in-situ methods were positively, but weakly correlated with each other ( $r^2=0.52$ ). Four tests to predict N mineralization potential were trialed: 24-h CO<sub>2</sub> burst, 7-day anaerobic incubation, 21-day aerobic incubation, and microbial biomass. None of the predictor tests correlated with plant uptake by broccoli. N mineralization in soil tubes was positively correlated with both 7-day anaerobic incubation ( $r^2=0.67$ ) and 21-day aerobic incubation ( $r^2=0.58$ ). Seasonal plant uptake fit a logistic curve while mineralization in soil tubes fit a single exponential equation.

Five out of eight of the farms produced marketable broccoli with no additional fertilizer. Overlaying the model for plant uptake with the model for N mineralization at one of the sites with relatively large N mineralization demonstrated that sufficient N was made available with no additional fertilizer with a mid-May planting of broccoli.

## Introduction

Increasing organic matter in soils is one way to build soil health (Doran and Zeiss 2000). Cover cropping, compost, and manure additions increase soil organic matter and improve the soil's ability to hold and supply nutrients, water, and air to plants and animals (Hargreaves, Adl, and Warman 2008; Evanylo et al. 2008; Oades 1984). Disturbed soils and soils low in organic matter can experience dramatic increases in productivity following organic matter addition (Carter 2002). Also, judicious use of organic amendments can maintain or enhance yields in soils with relatively high native productivity (Cogger et al. 2001; Evanylo et al. 2008).

Knowing the chemical composition of cover crop residue and other organic materials can help predict nitrogen (N) mineralization from these materials over the course of a season (Bending, Putland, and Rayns 2000; Gale et al. 2006). Nitrogen mineralization from soil organic matter has been widely studied. While N pools, processes, and functions have been described in general (Jarvis et al. 1996) the academic, consulting, and practicing agricultural community is still uncertain about crediting organic matter when preparing N fertilizer recommendations (Wienhold 2007). Crop fertilizer recommendations often credit a standard value for organic matter mineralization while soil laboratories and text books typically use the total soil organic matter to provide an estimated nitrogen release (ENR) value (Havlin et al. 2005; Brady and Weil 2002).

Efficient fertilization requires giving appropriate credit for the N mineralization potential of a soil. Predicting N mineralization is problematic because there is often poor correlation between net N mineralization (as estimated by laboratory incubation methods) and total soil organic matter content measured in soil tests (Jarvis et al. 1996). The lack of correlation between total organic matter and net N mineralization could be explained by the existence of distinct pools of organic matter which are not distinguished by values reported in a routine soil test. Decomposition of these two pools is governed by different rate constants with the first, "active" or "labile", pool decomposing at a more rapid rate than the second, "recalcitrant" pool. As noted by (Benbi and Richter 2002), many researchers have found that models that assume two distinct fractions are superior to those that assume only one. (Gilmour and Mauromoustakos 2011) proposed modeling nitrogen mineralization kinetics from the two pools as sequential instead of concurrent. Analyzing data from a broad range of soils the authors found a strong relationship between the mineralization rate during the first week of laboratory incubation ( $k_1$ ) and the nitrogen mineralized during that period (i.e. the "active" pool). In their study, three routine laboratory tests were effectively combined to predict  $k_1$ : total N, evolution of CO<sub>2</sub> after 3 days, and clay content.

Fast, simple, and cheap methods to predict N mineralization are desirable for farmers, especially those that rely heavily on soil building practices such as cover cropping and manure

for soil fertility. Certified organic farmers are encouraged to use these methods to build soil and are required to use only organic sources of nitrogen for fertility. Depending on N content of organic fertilizers and amendments, only small amounts of N may be made available in the year of application while the bulk is likely to contribute to the organic matter pool (Gale et al. 2006). Furthermore, soil properties across a single farm can be diverse, complicating generalizations and emphasizing the need for site-specific information (Collins et al. 2011).

(Sullivan, McQueen, and Horneck 2008) described a site-specific method for growers to estimate N mineralization potential by plant uptake over the course of a growing season using a “zero-N” plot. A more rapid method with the potential to predict N mineralization before the growing season is the Solvita™ colorimetric test for soils (Solvita Inc., Woods End, MA). The method has been correlated with other methods of determining carbon dioxide concentration (Haney et al, 2008). In addition to correlating 24-hour respiration with a 24-day incubation, Haney et al. (2001) also found strong correlation with forage uptake in fine sandy loam soil. The efficacy of these tests for predicting nitrogen mineralization has not been widely tested in field conditions, especially in western Washington.

## Objectives

The goals of the current study were to: 1) estimate the *in situ* seasonal nitrogen mineralized from soil on eight different certified organic farms in western Washington and 2) compare predicted values of N-mineralization via 24-hr CO<sub>2</sub> burst, 7-day anaerobic incubation, 21-day aerobic incubation, and soil chemical properties such as organic matter, soil C and soil N with measured N mineralization. *In situ* N-mineralization was estimated by plant uptake in a zero-N plot and by a buried soil method.

## Materials and Methods

*Site description and soil characteristics.* The geographic locations of the eight Washington State organic farms that cooperated in the study are shown in Figure 1. All sites, except for site 7, received some form of organic fertilizer or nitrogen-rich amendment in 2011. Management practices during 2007-2010 on all of the sites except site 5 included various combinations of manure, compost, and cover cropping. Site 5 was managed as a cattle pasture for at least a decade before 2011 and it was cropped to vegetables in 2011 (Table 1). All sites were located in western Washington with a maritime climate. Site 7 is the experimental organic farm at WSU Puyallup in Pierce County.

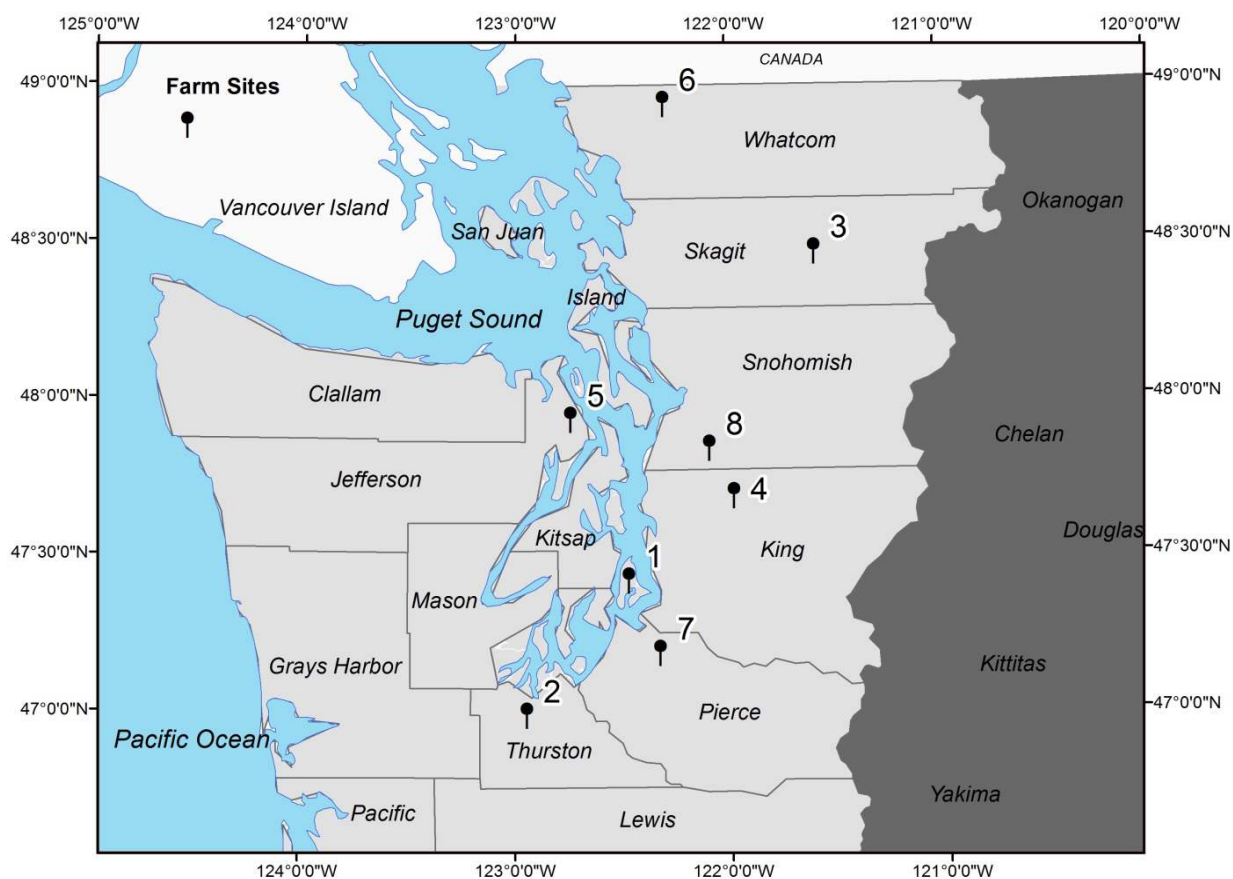


Figure 1. Location of eight organic farm sites in western Washington State.

Table 1. Previous management practices at eight cooperating organic farms in western Washington.

Site	Previous Management (2007-2010)	Fertilizer/ amendment in 2011
1	Cover crop, pastured poultry, and annual compost addition	Compost, fish-based organic fertilizer 7-7-7, kelp meal
2	Consistent winter cover cropping	1680 kg ha <sup>-1</sup> feather meal
3	Consistent winter cover cropping and annual spring application of organic fertilizer	620 kg ha <sup>-1</sup> 9-3-4 organic fertilizer
4	Consistent winter cover cropping	1120 kg ha <sup>-1</sup> 4-3-3 processed poultry manure

5	Grass hay and cattle	2240 kg ha <sup>-1</sup> 4-3-3
6	Yearly application of dairy manure	1120 kg ha <sup>-1</sup> 4-4-4 organic fertilizer
7	Compost applied 2010, 2008	None
8	Yearly chicken manure and compost	Chicken manure and compost

*Establishing zero-N plots.* The zero-N plots were located at each farm in March 2012 with flags at each corner. Plots were 18.5 m X 1.7 m allowing for planting two rows of broccoli and to provide enough space for 120 broccoli transplants with 0.3 m spacing within the row. Farmer cooperators were instructed to apply amendments (e.g. lime) as they would normally but omit any nitrogen containing fertilizers or amendments.

*Soil sampling and handling.* After establishing the location of the zero-N plots we collected 35 liters of soil from 0-30.5 cm depth by moving in a random pattern across the plot. At the time of collection the soils were too wet to sieve and repack into soil tubes so they were dried moderately by spreading out on a plastic tarp with circulating air at 10 C for 48-96 hours (as needed) with occasional stirring. The moist soil was then sieved to 9.5 mm. Sites 5 and 1 had 31% and 19% gravel content (g g<sup>-1</sup> soil) that did not pass through this size mesh and the proportion of removed material was used to adjust N-mineralization for these soils. After sieving, two subsamples were taken; one was kept refrigerated at 4 C until processing for microbial biomass and 21 day aerobic incubation and the other was dried in a soil dryer at 27C for 72 hours for the 24-hr CO<sub>2</sub> burst analyses and basic nutrient analysis.

*Soil chemical and physical parameters.* Bulk density was sampled at the same time that zero-N plots were established using 3 intact cores per plot collected with a hammer-driven core sampler (Gee and Or 2002). Total C and N were determined using a combustion analyzer equipped with an infrared detector (LECO Instruments Model CNS 2000, LECO Instruments, St. Joseph, MI)(Sweeney 1989). Organic matter was determined using the Walkley-Black method, and soil test P (Bray-1) and exchangeable K were determined using standard methods(Gavlak, Horneck, and Miller 2005). Soil nitrate-N and ammonium-N were determined by standard methods following extraction with 2 mol L<sup>-1</sup> KCl (Gavlak, Horneck, and Miller 2005). The soil pH was determined by preparing a 1:1 soil to water slurry; allowing samples to reach equilibrium at room temperature; and reading pH with a pH meter. Texture was determined with the hydrometer method of Gee and Or (2002).

*Mineralization in buried soils.* Soils were packed into 5.5 cm internal diameter PVC tubes (6.0 cm external diameter) that were cut to 20 cm lengths. In the bottom 1.3 cm of each tube we secured 30 grams (31 cm<sup>3</sup>) of anionic/cationic resin (65% A-464 XRR (OH) strong base gel anion resin and 35% USF C-211 (H) strong acid cation resin, Siemens Inc., Seattle, WA) in nylon mesh. Soils were then packed into the tubes at 0.95 of the field BD, except soil 4 which was packed at 0.9 field BD and soil 1 which was packed at field BD. Soil moistures at the time of packing were 0.28, 0.21, 0.29, 0.32, 0.18, 0.27, 0.24, 0.20 for soils 1-8, respectively.

The plastic tubes were inserted into the soil at the WSU Puyallup Research and Extension Center, Puyallup, WA. Soil cores of similar diameter to the external diameter of the tubes were removed and soil tubes were inserted into the holes on 21 March, 2012. Tubes were buried so that 1cm of the tube was above the soil line and exposed to ambient temperature and weather throughout the experiment. A nearby weather station (360 m away) provided air temperature and rainfall data. Growing degree days (GDD) were calculated from maximum and minimum air temperatures with a 0°C base temperature. A total of 2674 GDD were accumulated during the duration of the 182-day experiment. Three cores from each soil were removed every 2 weeks for analysis. Soils and resins were extracted separately with 2 mol L<sup>-1</sup> KCl and analyzed for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> (Gavlak, Horneck, and Miller 2005). For comparison to the plant uptake data, we estimated the amount of N mineralized per hectare multiplying by the site's bulk density. The final extractions were performed on 18 September, 2012.

*Plant uptake study.* All farmers were provided broccoli seeds (variety *Arcadia*) and instructed to start them and fertilize the starts with their own routine protocol. Farmers transplanted the broccoli between 4 May and 19 May 2012. At the time of transplanting farmers sent in 9 transplants for analysis. Every two weeks after transplanting farmers collected above-ground plant samples in a pattern similar to that described by Sullivan et al. (2008): three adjacent plants were combined into a single sample and 3 samples were collected (total of 9 plants). Plant packages were sent to our lab via overnight mail and after receiving packages plants were dried at 55 C and dry weight biomass was determined. Plants were then ground and analyzed for C and N. The final sample was intended to be harvest-ready broccoli heads. The fresh broccoli heads were evaluated for marketability at the terminal sample.

*Twenty-four-hour CO<sub>2</sub> burst.* Carbon dioxide evolution from dried and rewetted soil was determined with two different methods: 1) the commercially available Solvita Soil Life Test (Solvita Inc., Woods End, MA), and 2) using an infrared gas analyzer (IRGA). For both tests, 40 g of previously air-dried soil was placed on filter paper (Whatman GF/D, 4.25cm) in a 50 ml plastic beaker with 3 holes (3 mm diameter) in the bottom.

For the Solvita test, the beaker was then placed in a 250 ml glass air-tight container. The soil was wetted from the bottom with 25 ml deionized water. The Solvita kit provides a special paddle containing a CO<sub>2</sub> absorbent gel that changes color as it absorbs CO<sub>2</sub>. Jars were placed in a 25 C incubator and after 24 hours, paddles were read with a digital color reader (model S100, Solvita Inc.). The reader provides a color value between 0 and 5 and an estimated daily rate of CO<sub>2</sub> evolution (Haney, Brinton, and Evans 2008). One Solvita test was performed for each soil.

For the IRGA test, the plastic beakers were placed in a 244 ml glass jar. Before closing the lid, jars were flushed with ambient air and fitted with an air-tight lid that was equipped with a septum. Initial and final gas concentrations were determined according to a LI-COR Biosciences application note for measuring small volumes of CO<sub>2</sub> (LI-COR Biosciences 2013). The initial CO<sub>2</sub> concentration in each jar was recorded by removing 3 ml of gas from the jar immediately after sealing and the final CO<sub>2</sub> concentration was determined similarly after 24 hours. Four replications were performed for each soil. A standard curve was developed by injecting 3 ml of each CO<sub>2</sub> standard (0, 362, 2500, and 6000 ppm) into a constant stream of CO<sub>2</sub> free air that was

passed through the IRGA (LI-7000, LI-COR Biosciences, Lincoln, NE). The IRGA logs CO<sub>2</sub> concentration 50 times per second and calculates a peak integral. Three injections were made for each gas standard, and a standard curve was calculated based on the integral value.

*7-day lab anaerobic incubation.* Mineralized nitrogen as extractable NH<sub>4</sub>-N was determined after anaerobically incubating 20 g of air-dried ground soil with 25 ml of deionized water at 40 C for 7 days (soil nitrogen mineralization potential anaerobic method (Gavlak, Horneck, and Miller 2005)).

*Microbial biomass.* Microbial biomass was determined using a modification of the substrate induced respiration (SIR) method described by Horwath and Paul(1994). The modified method allowed for continual purging of the sample jar in a fashion similar to that described by Heinemeyer et al.(1989). The equivalent of 10 g oven dry, pre-incubated soil (adjusted to 40 to 50% water holding capacity and incubated at 25°C for 16 h) was dispersed to evenly cover the bottom of a 244 mL canning jar and 4 replicates were performed for each sample. Glucose solution was added with a syringe fitted with a 1.3-cm 27-gauge needle to bring the soil water content to 75% water holding capacity and the glucose concentration to 20 µmol glucose g<sup>-1</sup> soil solution. Jars were closed with an air-tight metal lid fitted with two Super Speedfit tube connections (US Plastic Corporation, Lima, OH). Bev-a-line (6.35 mm outer diameter) was connected from the intake tube connector to a 3-way ball valve (John Guest Connection, US Plastic Corporation). During the experiment, the ball valve was turned to allow humidified ambient air to pass through the jar and exit through the second tube connector. Based on preliminary studies we have found 2 hours to be the optimal time for maximum initial respiration. At two hours after introducing glucose, the tube from the jar outtake was connected to the IRGA intake and the jar intake was connected to the IRGA outtake forming a closed cell. The internal IRGA pump circulated air through the chamber and CO<sub>2</sub> concentration was logged once per second for 90 seconds. The initial 30 seconds were disregarded and the final 60 seconds were used to determine a rate of CO<sub>2</sub> evolution. Microbial biomass was expressed as ml CO<sub>2</sub> 100g<sup>-1</sup> soil hr<sup>-1</sup>.

*21-day aerobic incubation.* Nitrogen mineralization over a 21-day period was determined by placing 500 g of moist soil in zippered 3.8-L polyethylene bags in a procedure adapted from Gale et al(Gale et al. 2006). Soils had the same soil moisture content as when tubes were packed for the mineralization study. Bags were partially sealed and a straw inserted to allow air exchange. Bags were placed in an incubator at 35 C. Initial and final nitrate and ammonium concentrations were determined as described above (Gavlak, Horneck, and Miller 2005).

*Statistical analyses.* Nitrogen uptake by broccoli at each site was fit to a logistic equation:

$$N = \frac{a}{1+e^{(b-ct)}} \quad [1]$$

Where  $N$  = nitrogen accumulation in kg N ha<sup>-1</sup> at days after transplanting  $t$ ,  $a$  is the maximum nitrogen accumulated in kg N ha<sup>-1</sup>,  $b$  the initial nitrogen accumulated in kg N ha<sup>-1</sup>, and  $c$  is an uptake constant representing the rate of N accumulation per day at the onset of the growth period in kg N ha<sup>-1</sup> day<sup>-1</sup> (Moustakas and Ntzanis 2005; Spiegelman 1946).

N mineralization from buried soil tubes were fit to a single exponential equation:

$$N_t = N_0 [1 - e^{-kt}] \quad [2]$$

Where  $N_t$  = the amount of N mineralized in  $\text{mg kg}^{-1}$  at the accumulated growing degree days (GDD)  $t$ , and  $N_0$  represents the amount of mineralizable substrate in one season in  $\text{mg kg}^{-1}$  soil.

Estimates of the parameters of the nonlinear model functions were fit by weighted least-squares using the *nls* function in the R software package and the 95% confidence interval for each parameter was computed (R Development Core Team 2011).

We also used R to calculate a correlation matrix for the potential predictor variables, measured N uptake and *in situ* mineralization and parameters of the logistic equation for modeled N uptake ( $a$  and  $c$ ) and modeled mineralization in buried soil tubes ( $N_0$  and  $k$ ). After selecting the date from each site with the largest mean for N plant uptake and maximum N mineralization in tubes, farm sites were separated by student's t-test ( $\alpha=0.05$ ) following Kruskal-Wallis analysis of variance by ranks ( $n=3$ ) with the R package *agricolae* (Mendiburu 2010). This nonparametric test was used since the dates for the analysis were selected and were therefore not random or independent.

## Results

Soil geographic, chemical, and physical parameters at the eight sites are summarized in Tables 2 and 3. Soil texture ranged from sand to silt loam, with no soil containing more than 15% clay. Soil C ranged from 17 to 40  $\text{g kg}^{-1}$  (Table 3).

*Tests to predict N-mineralization potential.* The rate of  $\text{CO}_2$  evolved over a 24-hour period from a dried and rewetted soil ranged from 34 to 85  $\text{mg CO}_2 \text{ kg}^{-1} \text{ soil}^{-1} \text{ day}^{-1}$  when measured with the IRGA and from 31 to 60  $\text{mg CO}_2 \text{ kg}^{-1} \text{ soil}^{-1} \text{ day}^{-1}$  when measured with the Solvita colorimetric test. The two tests were highly correlated ( $r^2 = 0.90$ ) though the Solvita tended to underestimate  $\text{CO}_2$  as compared to the IRGA (Table 4). The  $\text{NH}_4\text{-N}$  mineralized in a 7-day anaerobic incubation ranged from 9 to 25  $\text{mg kg}^{-1}$  and  $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$  mineralized in a 21-day aerobic incubation ranged from 37 to 71  $\text{mg kg}^{-1}$ . Microbial biomass estimated with substrate-induced respiration ranged from 0.83 to 1.43  $\text{ml CO}_2 100 \text{ g}^{-1} \text{ hr}^{-1}$  (Table 4).



Table 2. Altitude and selected soil physical properties at eight cooperating organic farms in March 2012.

Site	Sand (%)	Silt (%)	Clay (%)	Texture Class	Mapped Soil Series	Soil Taxonomy	BD g cm <sup>-3</sup>	WHC %	Altitude m
1	66	28	6	Gravelly sandy loam	Alderwood	Aquic Dystrochrepts	0.89	62	87.5
2	88	10	3	Sand	Nisqually	Pachic Humixerepts	1.15	58	58.9
3	40	49	12	Loam	Larush	Vitrantic Humixerepts	1.04	70	70.8
4	24	61	15	Silt loam	Nooksack	Fluventic Haploxerolls	1.16	68	10.5
5	69	24	7	Gravelly sandy loam	Belfast	Aquandic Xerofluvents	1.20	38	69.0
6	36	52	12	Silt loam	Mt. Vernon	Aquic Vitrixerands	1.01	51	24.1
7	58	29	12	Sandy loam	Puyallup	Fluventic Haploxerolls	1.13	47	9.7
8	42	47	12	Loam	Puyallup	Fluventic Haploxerolls	1.19	51	7.6

BD=Bulk density; WHC=Water holding capacity.

Table 3. Selected soil chemical properties at eight cooperating organic farms in March 2012.

Site	OM g kg <sup>-1</sup>	Total C g kg <sup>-1</sup>	Total N g kg <sup>-1</sup>	C:N	NO <sub>3</sub> -N mg kg <sup>-1</sup>	NH <sub>4</sub> -N mg kg <sup>-1</sup>	P mg kg <sup>-1</sup>	K mg kg <sup>-1</sup>	pH
1	88	40	3.2	12.6	10.9	2.7	65	113	6.4
2	128	36	3.0	12.3	5.5	3.1	100	95	6.6
3	56	24	2.2	10.7	3.8	1.4	201	63	6.0
4	48	20	2.1	9.7	3.6	1.1	21	70	5.9
5	52	20	1.8	11.3	11.5	2.6	35	263	5.8
6	40	19	1.9	9.9	4.0	1.5	22	188	6.2
7	40	18	1.8	9.8	2.6	2.5	420	165	5.8
8	36	17	1.5	11.0	3.9	1.8	135	128	6.3

OM= Organic matter.

Table 4. Twenty-four hour CO<sub>2</sub> burst, 7-day anaerobic incubation, 21-day aerobic incubation, and microbial biomass from eight cooperating organic farms in March 2012.

Site	IRGA 24h mg CO <sub>2</sub> kg <sup>-1</sup> soil <sup>-1</sup> day <sup>-1</sup>		Solvita 24h mg CO <sub>2</sub> kg <sup>-1</sup> soil <sup>-1</sup> day <sup>-1</sup>		7-Day Anaerobic Incubation	21-Day Aerobic Incubation			Microbial Biomass		
					NH <sub>4</sub> -N mg kg <sup>-1</sup>	mg NO <sub>3</sub> -N + NH <sub>4</sub> - N kg <sup>-1</sup>			ml CO <sub>2</sub> 100g <sup>-1</sup> hr <sup>-1</sup>		
1	67.6	± 2.9	47.8		24.6	70.8	± 3.4		1.43	± 0.21	
2	33.8	± 4.8	30.7		25.2	61.5	± 6.0		1.43	± 0.15	
3	71.7	± 2.2	47.8		12.9	52.9	± 3.8		1.15	± 0.16	
4	80.1	± 9.4	59.5		11.7	40.5	± 4.1		0.76	± 0.06	
5	55.4	± 3.5	48.6		19	36.7	± 3.6		0.86	± 0.06	
6	39.1	± 3.6	33.1		8.7	46.1	± 2.9		0.83	± 0.04	
7	52.7	± 4.1	44.7		13.5	54.0	± 11.5		1.42	± 0.26	
8	84.9	± 2.8	59.5		11.9	42.2	± 2.5		1.13	± 0.36	

IRGA=Infrared gas analyzer. Values are ± standard deviation where measurement was replicated: IRGA, n=4; 21-day aerobic incubation, n=3; microbial biomass, n=4.

*In situ measurements of nitrogen mineralized from organic matter.* In the plant uptake study, the maximum nitrogen accumulation ranged from 42 to 131 kg N ha<sup>-1</sup> across the 8 sites. Sites 1, 3 and 7 had N uptake equal to or exceeding 130 kg ha<sup>-1</sup> and sites 4 and 5 had N uptake of 105 and 114 kg ha<sup>-1</sup>, respectively. These five farms all produced acceptable, marketable fresh weights of broccoli in the zero-N plots, while the other 3 farms did not produce large enough heads to be marketable. Using non-linear regression we fit logistic curves to all sites except for 3, 4, and 8 (Figure 2). Sites 3 and 8 did not reach a plateau with the number of samples collected. The final sample date for site 4 indicated a significant drop in N accumulation compared to the previous sample; though we could fit a logistic curve the asymptote parameter,  $a$ , was not significant.

Daily minimum and maximum temperatures, daily precipitation, and soil moisture within tubes at each sampling date are shown in Figure 3. Tubes were exposed to seasonal precipitation for the duration of the experiment and were irrigated 5 times in the month of August. A total of 2674 GDD were accumulated during the duration of the 182-day experiment.

In the buried soil tube study the range of maximum N mineralization measured was 25 to 53 mg NO<sub>3</sub>-N + NH<sub>4</sub>-N kg soil<sup>-1</sup> (Table 5). All of the curves were fit to single exponential equations and the range of  $N_0$ , the modeled size of the seasonal mineralizeable pool was 26 to 56 mg kg soil<sup>-1</sup> (Figure 4, Table 5). The modeled size of the pool correlated well with the observed maximum value of N mineralized ( $r=0.92$ , Table 6). Using the bulk density at each site we estimated maximum mineralization to be 91 to 156 kg NO<sub>3</sub>-N + NH<sub>4</sub>-N ha<sup>-1</sup> (Table 5).

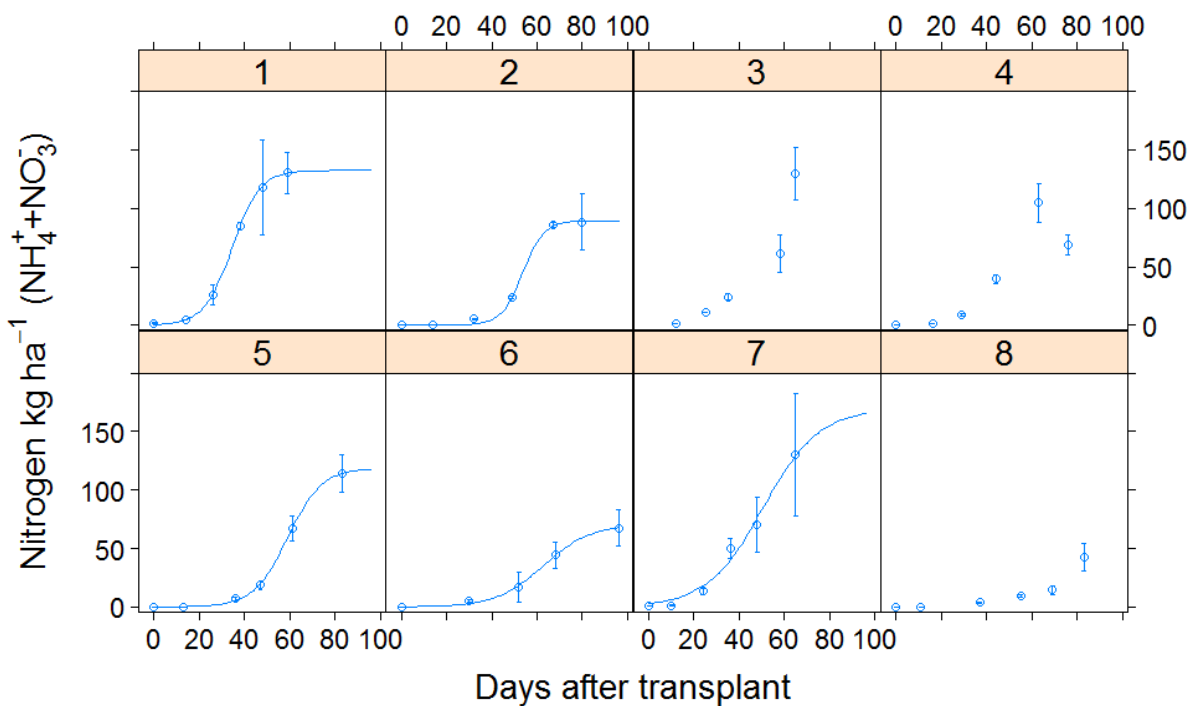


Figure 2. Total nitrogen uptake in broccoli plants from plots that received no nitrogen fertilizer or amendments at 8 organic farms in western Washington in 2012. Bars are SD,  $n=3$ .

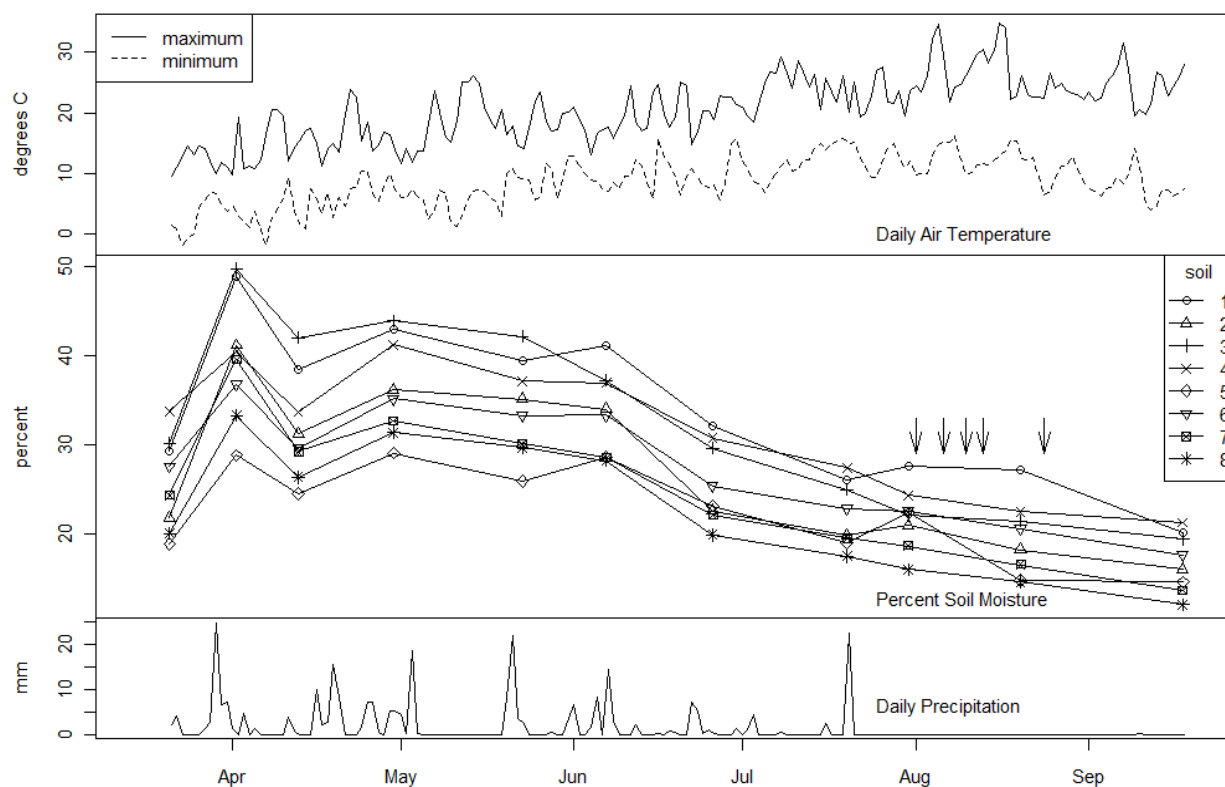


Figure 3. Maximum and minimum air temperature, daily precipitation, and average percent soil moisture in buried soil tubes ( $n=3$ ). Arrows indicate irrigation events.

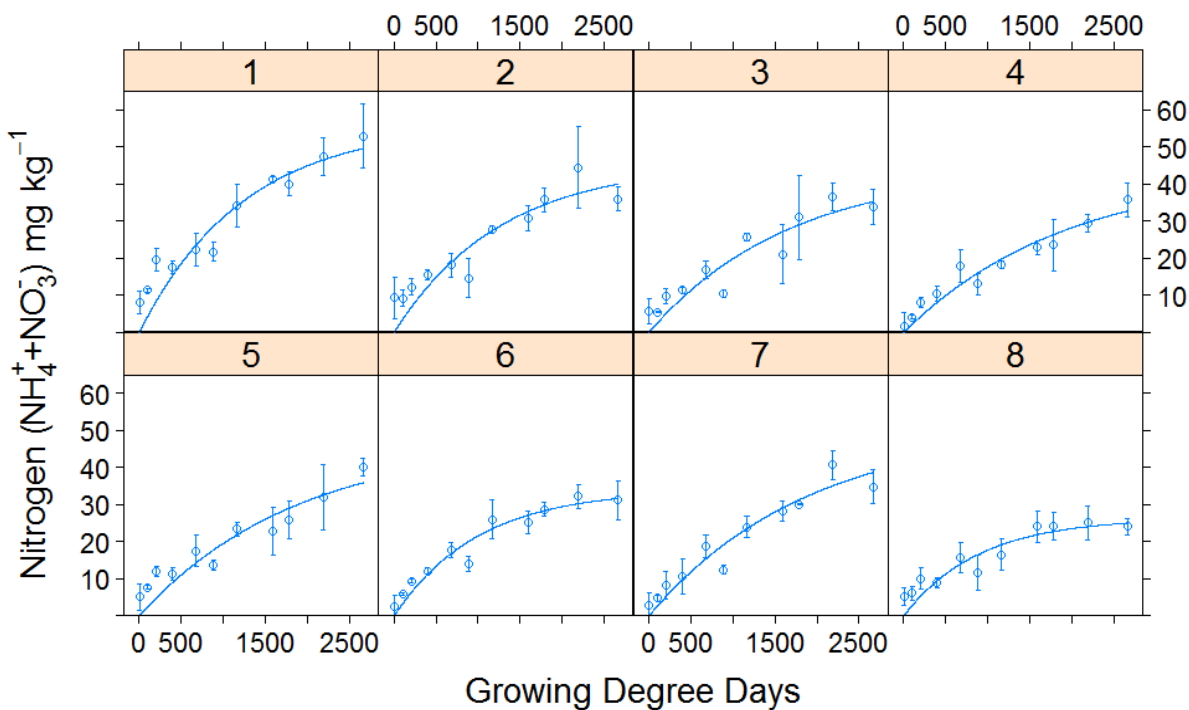


Figure 4. Total nitrogen mineralized from buried soil tubes from 8 organic farms in western Washington in 2012. Bars are SD,  $n=3$ .

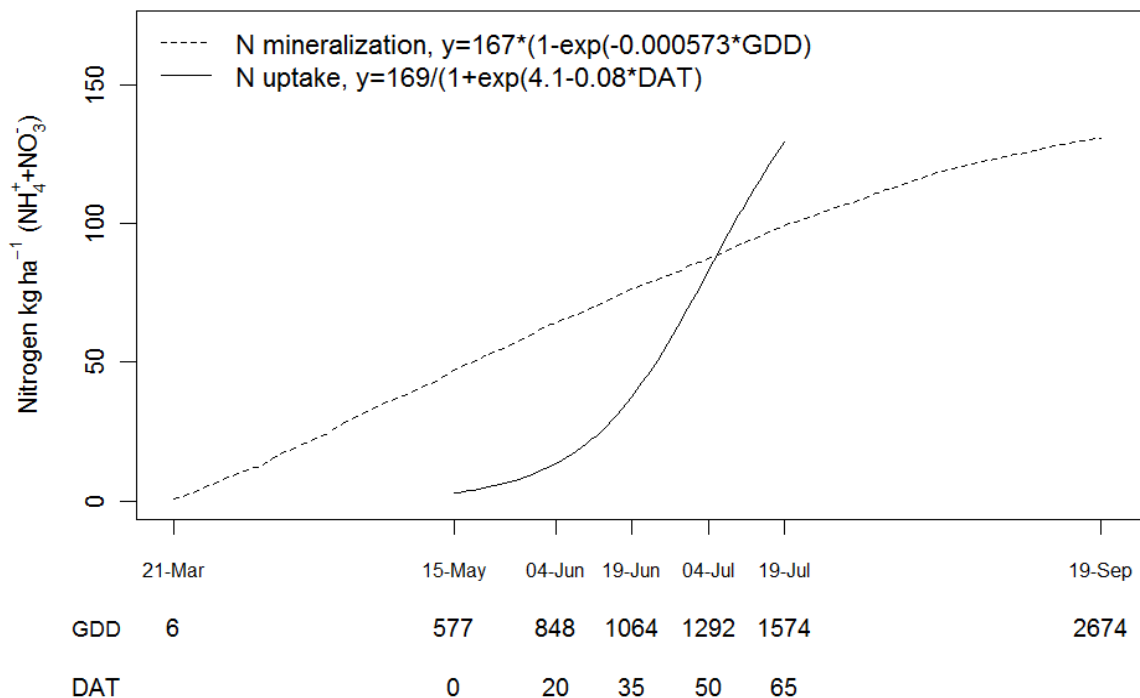


Figure 5. Nitrogen uptake by broccoli and nitrogen mineralization from buried soil tubes for site 7 (WSU Puyallup Research and Extension Center). Mature broccoli heads were harvested 65 days after transplant. GDD=Cumulative growing degree days, base= $0^{\circ}\text{C}$ ; DAT = Days after transplant.

Site 7 was the WSU-Puyallup Research and Extension Center experimental organic farm. This was also the location of the buried soil study which allowed direct comparison between modeled nitrogen mineralization and plant uptake (Figure 5). The buried soil study was started on 21 March 2012, and plants were transplanted on 15 May 2012. For the purpose of Figure 5, N mineralization began on 21 March 2012, which is the same parameter used to develop the mineralization model. This indicated a surplus of available N relative to the plant needs at the time of transplanting. As plant growth rate and N uptake increased after the first inflection, uptake rate surpassed mineralization rate. Fitted plant uptake was 129 kg N ha<sup>-1</sup> at the time of initial harvest (19 July) compared to 99 kg ha<sup>-1</sup> mineralized by that date.

*Coefficients of correlations.* Table 6 shows the coefficients of correlations (*r*) between potential predictors of N-mineralization, selected soil chemical properties, and measured and modeled N-uptake by broccoli and N-mineralized in soil tubes. The two *in situ* tests designed to estimate actual N-mineralization, N-uptake by broccoli and N-mineralization in soil tubes, were positively correlated (*r*=0.72). Measured N-uptake was also correlated with modeled maximum uptake, *a*, (*r*=0.93) and with the modeled mineralizeable pool, *N<sub>0</sub>* (*r*=0.92) and the modeled accumulation rate, *k* (*r*=-0.82). The modeled uptake constant *c*, the rate of N accumulation per day at the onset of the growth period, was correlated with 7-day anaerobic mineralization, soil OM, and C:N. However, measured N-uptake and the modeled maximum N-uptake, *a*, were not significantly correlated with soil chemical properties or predictor tests. On the other hand, the maximum measured values of N-mineralization from soil tubes were correlated with 7-day anaerobic incubation (*r*=0.82), 21-day aerobic incubation (*r*=0.76), total N (*r*=0.84) and total C (*r*=0.83). Correlations between these tests and the modeled values of the size of the mineralizeable pool, *N<sub>0</sub>*, were weaker and insignificant at *p*<0.05. Neither test of 24-hr CO<sub>2</sub> evolution was correlated with measured or modeled values on N-uptake or mineralization. There was also a lack of correlation between 24-hr CO<sub>2</sub> evolution and soil chemical properties.

Table 5. Maximum N uptake by broccoli, maximum N mineralized in soil tubes, logistic model parameters for N uptake, and single exponential model parameters for N mineralization from soil tubes.

Site	N Uptake by Broccoli				N Mineralization from Buried Soil Tubes			
	Maximum measured kg N ha <sup>-1</sup>	Parameters of logistic equation			Maximum measured <sup>†</sup> kg N ha <sup>-1</sup> mg N kg <sup>-1</sup>	Parameters of exponential equation		
		<i>a</i> ± 95% CI kg N ha <sup>-1</sup>	<i>b</i> ± 95% CI kg N ha <sup>-1</sup> day <sup>-1</sup>	<i>c</i> ± 95% CI kg N ha <sup>-1</sup>		<i>N</i> <sub>0</sub> ± 95% CI mg N kg <sup>-1</sup>	<i>k</i> ± 95% CI mg N kg <sup>-1</sup> GDD <sup>-1</sup>	
1	131 a <sup>‡</sup>	132 ± 22	5.6 ± 2.9	0.16 ± 0.09	144	53 a <sup>‡</sup>	56 ± 14	7.7 X 10 <sup>-4</sup> ± 3.92 X 10 <sup>-4</sup>
2	88 bcd	89 ± 12	11.4 ± 9.3	0.21 ± 0.19	156	45 ab	46 ± 14	7.9 X 10 <sup>-4</sup> ± 4.74 X 10 <sup>-4</sup>
3	130 a	dnc	dnc	dnc	116	37 bc	44 ± 19	5.9 X 10 <sup>-4</sup> ± 4.54 X 10 <sup>-4</sup>
4	105 abc	ns	14.1 ± 75.3	0.32 ± 1.71	127	36 bcd	44 ± 17	5.2 X 10 <sup>-4</sup> ± 3.22 X 10 <sup>-4</sup>
5	114 ab	119 ± 12	7.8 ± 2.2	0.13 ± 0.04	147	40 ab	47 ± 21	5.3 X 10 <sup>-4</sup> ± 3.99 X 10 <sup>-4</sup>
6	67 cd	70 ± 16	6.0 ± 4.0	0.10 ± 0.07	99	32 cd	34 ± 5	10.1 X 10 <sup>-4</sup> ± 3.58 X 10 <sup>-4</sup>
7	130 ab	169 ± 146	4.1 ± 2.2	0.08 ± 0.08	140	41 ab	51 ± 19	5.7 X 10 <sup>-4</sup> ± 3.16 X 10 <sup>-4</sup>
8	42 d	dnc	dnc	dnc	91	25 d	26 ± 5	11.2 X 10 <sup>-4</sup> ± 5.58 X 10 <sup>-4</sup>

GDD= growing degree days; dnc= did not converge; ns= not significant; CI=Confidence Interval. <sup>†</sup>kg N ha<sup>-1</sup> was estimated in the mineralization study by accounting for soil bulk density at each site. <sup>‡</sup>Means with the same letter are not significantly different at *p*<0.05 by student's t-test following Kruskal-Wallis analysis of variance by ranks (n=3).

Table 6. Coefficients of correlations between N-mineralization predictor tests, soil chemical properties, and measured and modeled N-uptake and N-mineralization in soil tubes.

	IRGA 24h	Solvita 24 h	7Day an- aerobic	MicBio	21Day aerobic	OM	Total N	Total C	C:N	Max N uptake	Max N min	Uptake model $a^{\dagger}$	Uptake model $c^{\dagger}$	N-min model $N_0$
Solvita 24 h	0.95**													
7Day anaerobic	-0.30	-0.30												
MicBio	-0.20	-0.30	0.60											
21Day aerobic	-0.26	-0.42	0.65	0.83*										
OM	-0.45	-0.53	0.87**	0.56	0.69									
Total N	-0.28	-0.40	0.81*	0.54	0.85**	0.88**								
Total C	-0.26	-0.38	0.88**	0.62	0.86**	0.90**	0.98**							
C:N	-0.14	-0.23	0.91**	0.57	0.64	0.79*	0.74*	0.85**						
Max N uptake	-0.02	-0.01	0.34	0.30	0.40	0.17	0.39	0.33	0.10					
Max N min	-0.33	-0.34	0.82*	0.55	0.76*	0.68	0.84**	0.83*	0.62	0.72*				
Uptake model $a^{\dagger}$	0.66	0.74	0.09	0.52	0.19	-0.26	-0.13	-0.09	-0.10	0.93*	0.42			
Uptake model $c^{\dagger}$	-0.18	-0.30	0.89*	0.41	0.50	0.97**	0.83	0.85	0.90*	-0.09	0.60	-0.34		
N-min model $N_0$	-0.20	-0.16	0.62	0.44	0.57	0.43	0.61	0.58	0.34	0.92**	0.92**	0.79	0.24	
N-min model $k$	-0.02	-0.14	-0.12	0.06	0.08	-0.00	-0.05	-0.03	0.22	-0.82**	-0.40	-0.78	0.17	-0.70

IRGA=infrared gas analyzer, MicBio=substrate-induced respiration microbial biomass, OM=organic matter, Max N uptake = maximum measured value for N uptake by broccoli; Max N min= Maximum measured value for N mineralized in soil tube study; Uptake model  $a$  and  $c$  are fitted parameters for logistic equation for N uptake by broccoli; N-min model  $N_0$  and  $k$  are fitted parameters for single exponential model for N-mineralization from soil tubes. \*= $p<0.05$ , \*\*= $p<0.01$ .  $^{\dagger}$  n=5 for uptake model parameters and n=8 for all other correlations.

## Discussion

One goal of this study was to estimate the *in situ* seasonal nitrogen mineralized from soil on eight different certified organic farms in western Washington. In other words, have historic soil building practices provided inherent soil fertility that can significantly reduce the need for supplemental fertilizers? A second goal of the study was to evaluate the N-mineralization predictive capability of 24-hr CO<sub>2</sub> burst, 7-day anaerobic incubation, 21-day aerobic incubation, and soil chemical properties such as organic matter, soil C and soil N.

Nitrogen mineralization varied significantly across study sites; the spread between the site with the largest N mineralization and the site with the smallest N mineralization was 88 kg N ha<sup>-1</sup> in the plant uptake study and 65 kg N ha<sup>-1</sup> in buried soil tube study. The farms in this study with the largest N mineralization could likely apply much less fertilizer than those with less N mineralization potential. A follow-up study that compared fertilizer application rates on soils with a history of soil building practices would be needed to define the return on investment from annual fertilizer application on these soils. Not only can historic amendments affect N mineralization rate from organic matter, they can also affect the availability of recently added N sources. For example, Mallory and Griffin (2007) found that historically amended soils provided more N from organic matter than unamended soils but were also more likely to immobilize supplemental N.

Honeycutt(1999) used *in situ* mineralization tubes to study mineralization from soil and vetch. The soil used for the study, a Spodosol from Newport, ME, had not been fertilized for 2 years before sampling. Buried soil tube studies were performed in 1993 and 1994; only 29 mg N kg<sup>-1</sup> were mineralized after 2500 GDD in 1993 and 20 mg N kg<sup>-1</sup> mineralized following the same number of GDD in 1994. The only soil from our study that was in this range was soil 8, which mineralized 25 mg N kg<sup>-1</sup> (after 2674 GDD; 24.4 mg N kg<sup>-1</sup> at 2500 GDD based on Equation 1). All other soils exceeded this amount, with soil 1 doubling the N mineralization observed by Honeycutt (1999).

We found a significant but weak correlation between the two *in situ* methods for evaluating mineralization (plant uptake and buried soil tubes), based on the maximum value from each ( $r^2=0.52$ ). Interestingly, maximum plant uptake was not correlated with any of the other predictor tests we evaluated, though N mineralization was cross-correlated with other tests. Similarly, Wienhold (2007) found that buried soil tubes were the best estimator of plant uptake. N uptake in cool-season vegetables is affected by both temperature and light (Sanchez and Doerge 1999), variables that were not recorded at the on-farm sites. Though the sites all share the same general maritime climate, there are likely significant microclimate differences resulting from differences in altitude and distance from Puget Sound. The inclusion of weather monitoring instruments at each farm site could improve interpretation of the uptake data (Wurr, Fellows, and Phelps 2002). Farm management, such as soil preparation and irrigation, was not controlled and these also affect plant growth, N mineralization, and uptake.

The only test to correlate with plant uptake was *in situ* mineralization in buried soil tubes. Both 7-day anaerobic incubation and 21-day aerobic incubation were correlated with the maximum measured N mineralization from soil tubes ( $r=0.82$  and  $0.76$ , respectively). Surprisingly there



was no correlation with either test used to estimate 24-h CO<sub>2</sub> burst and any of the soil parameters or estimates of plant uptake (Table 6). Franzluebbers et al. (2000) found a strong correlation between 1-day and 3-day CO<sub>2</sub> evolution. They subsequently found strong correlations between 3-day CO<sub>2</sub> evolution and 24-day mineralization, soil microbial biomass, particulate organic C, and soil organic C. We did find a correlation between microbial biomass and mineralization during the 21-day aerobic incubation ( $r=0.83$ ), but microbial biomass was not correlated to N uptake by broccoli or N mineralization in soil tubes. Gilmour and Mauromoustakos (2011) combined 3-day CO<sub>2</sub>, total N, and clay content in a multiple linear regression to accurately predict values of  $k_f$  (rate constant for the active carbon pool). The combined importance of these factors indicates that the relationship between CO<sub>2</sub> evolution and mineralization rate is affected by clay content and the total size of the N pool. While the relationship between laboratory mineralization and CO<sub>2</sub> evolution may be strong for a group of similar soils, the relationship can differ between soils (Franzluebbers et al. 2000). With only 8 soils representing diverse soil types our ability to find a relationship between N mineralization and prediction tests was reduced. Using the same 24-hr CO<sub>2</sub> burst method we have been able to detect significant differences between treatments from the same soil type in a long-term organic farming systems experiment at WSU-Puyallup (McCann et al. 2011).

Plants have different rates of nutrient uptake during their development and the change in uptake rate through a season can be described by an S-shaped logistic equation (Moustakas and Ntzanis 2005; Sanchez and Doerge 1999). When the model for seasonal N mineralization in soil 7 was superimposed on the plant uptake curve at the same site it is evident that the N mineralization rate exceeds plant N uptake early in the season. Transplanting in mid-May was well timed to take advantage of early season N mineralization, assuming the pool of accumulated N was not lost to leaching. Following the first inflection in modeled uptake, the rate of plant uptake increased to a rate surpassing the mineralization rate. Broccoli transplanted earlier in the spring might have experienced N stress during the period of maximum uptake and yielded less or required more time to mature.

## Conclusions

Soil building practices such as cover cropping and addition of compost or manure build soil organic matter. Soil organic matter provides a bank of nutrients, including nitrogen, that are made available through mineralization. Our study did not include enough farm sites or have sufficiently detailed plot histories to completely describe how previous management interacts with soil type to provide plant-available nitrogen from organic matter. However, we found that most of the plots on organic farms in our experiment did mineralize sufficient nitrogen to produce marketable broccoli heads. Only farms 4, 6, and 8 did not produce marketable head weights. These farms also yielded among the lowest nitrogen uptake and nitrogen mineralization, though only site 8 separated ( $p<0.05$ ) from the other sites. Several participating farmers commented that they were surprised to see how well their broccoli grew in the zero nitrogen plots without additional fertilizer.

On organic farms nitrogen not mineralized from organic matter, cover crops, or amendments must be supplied through certified organic fertilizers. These fertilizers are expensive sources of N, ranging from approximately \$10 to \$18 per kg N. For the farmer that can reliably reduce N

inputs this represents a savings of several hundred dollars per ha. Determining how much organic fertilizer to apply is a delicate process; too much fertilizer is a potential waste of money and can lead to nitrogen leaching while too little fertilizer will not optimize yield. This indicates the need for site-specific information for farmers wishing to make efficient fertilizer applications. In our study, 7-day anaerobic incubation, 21-day aerobic incubation, total N, and total C provided the strongest correlation with *in situ* mineralization of nitrogen. None of the predictors, however, correlated with plant uptake. The on-farm sites were exposed to varying soil and crop management, both of which influence how laboratory soil N mineralization predictions are actually realized (Honeycutt 1999). On-farm, plant N uptake studies are valuable because they directly measure what a rapid lab test is attempting to predict. The ability to integrate soil and climatic conditions *in situ* strengthens the applicability of the findings but also may add noise to the results and confound the ability to draw general conclusions. Monitoring light intensity and temperature in conjunction with N mineralization kinetics at remote sites could strengthen models and our understanding of how crop uptake relates to potential N mineralization in terms of magnitude and synchronicity.

## Outreach

Two Extension articles about the project were published and are included in the addenda:

Collins, D.P. , C.G. Cogger, and A.I. Bary. 2013. Seasonal nitrogen mineralization on organic farms. *Tilth Producers Quarterly*. 23 (1): 1,4-5.

Collins, D.P. 2012. Northwest soil science: nitrogen mineralization. *Read the Dirt*. [online] <http://readthedirt.org/northwest-soil-science-nitrogen-mineralization>

Also, a presentation was given at the Tilth Producers of Washington annual conference in Port Townsend, WA, November 2012: *Predicting N mineralization from organic matter*. Approximately 100 organic growers attended the presentation.

An academic publication was submitted to *Renewable Agriculture and Food Systems* in May 2013.

## Leveraged Resources

A USDA OREI grant helped support this project: Designing Production Strategies for Stewardship and Profits on Fresh Market Organic Farms, Grant # 2008-01247. We are also planning to leverage this work in a future grant

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## Photos and Addenda

I. Northwest Soil Science: Nitrogen Mineralization – Read the Dirt.pdf  
Article written for the online publication, “Read the Dirt.” [online]  
<http://readthedirt.org/northwest-soil-science-nitrogen-mineralization>

II. 23\_1Collins.pdf  
Article written for Tilth Producers Quarterly, a print journal for members of the organic farming group, Tilth Producers of Washington. Available online for members: [www.tilthproducers.org](http://www.tilthproducers.org)

III. SampleFarmerLetter.pdf  
Letters describing the farmer-cooperator role in the experiment were sent to each farmer.

- IV. Photos.
- a. IMG\_0575.jpg, PI Doug Collins soil sampling at cooperating farm, Growing Washington, Everson, WA. Photo by Clayton Burrows.
  - b. IMG\_0582.jpg, PI Doug Collins soil sampling at cooperating farm, Growing Washington, Everson, WA. Photo by Clayton Burrows.
  - c. IMG\_0595.jpg, Cooperating farmer Clayton Burrows, Growing Washington, Everson, WA, assisting with soil sampling. Photo by Doug Collins
  - d. IMG\_0598.jpg, PI Doug Collins taking bulk density samples with farmer cooperators Adam Brown and Haley Brown of Chinook Farm, Snohomish, WA.
  - e. IMG\_2349.jpg, Broccoli plants growing in a zero-nitrogen plot at Plum Forest Farm, Vashon Island, WA. Photo by Rob Peterson, farmer participant.
  - f. IMG\_2352.jpg, Mature broccoli ready for harvest from Plum Forest Farm, Vashon Island, WA. Photo by Rob Peterson.
  - g. IMG\_6353.jpg, Preparing for buried soil tube study, Puyallup WA. Co-PI Andy Bary (left) and Liz Myhre (right). Photo by Doug Collins
  - h. IMG\_6362.jpg, Inserting soil for buried soil tube study, Puyallup WA. Co-PI Andy Bary (right) and Liz Myhre (left). Photo by Doug Collins
  - i. IMG\_6364.jpg, Tubes for the buried soil tube study. Exchange resins to trap leached nutrients are secured to the bottom of the tube. Photo by Doug Collins
  - j. IMG\_6365.jpg, Tubes inserted into soil for buried soil tube study. Photo by Doug Collins
  - k. IMG\_6411.jpg, Co-PI Andy Bary preparing soil from buried soil tube study for chemical extraction. Photo by Doug Collins
  - l. IMG\_6514.jpg, Preparing soil for 24-hr CO<sub>2</sub> burst test. Photo by Doug Collins.