



# ORGANIC FARMING RESEARCH FOUNDATION

*Project report submitted to the Organic Farming Research Foundation:*

**Project Title:**

***Brassicaceae Seed Meal Application for Weed Reduction and Improved Nitrogen Management in Organic Farming Systems***

FINAL PROJECT REPORT

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**Principal investigator:**

Jodi Johnson-Maynard  
Soil & Land Resources Division  
Dept. of Plant, Soil and Entomological Sciences  
University of Idaho  
Moscow, ID 83844  
208-885-6245  
[jmaynard@uidaho.edu](mailto:jmaynard@uidaho.edu)

**Project participants:**

Matthew Morra, Soil & Land Resources Division, Dept. of Plant, Soil and Entomological Sciences, University of Idaho  
Louise-Marie Dandurand, Soil & Land Resources Division, Dept. of Plant, Soil and Entomological Sciences, University of Idaho  
Cinda Williams, Sustainable Agriculture Coordinator, Dept. of Plant, Soil and Entomological Sciences, University of Idaho  
Mary Jane Butters, Paradise Farm Organics, Moscow, ID

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## Project Summary

Soil amendments used in organic farming may limit N availability and can be cost prohibitive. Mechanical weed control can destroy soil aggregation thereby increasing runoff and erosion. Our objective was to determine if Brassicaceae seed meal, a byproduct of oil extraction that contains allelochemical-producing compounds called glucosinolates, can increase plant-available N and yields of lettuce (*Lactuca sativa*) and beets (*Beta vulgaris*). Additionally we tested the ability of meal to control weeds and its influence on crop seed germination. Three seed meals with varying glucosinolate concentrations and profiles, *Brassica juncea* (BJ), *Brassica napus* (BN), and *Sinapis alba* (SA), were amended to field soils at two different rates (3 and 10 Mg ha<sup>-1</sup>) in each of two years. Crop yields and N uptake varied with precipitation, meal type, and amendment rate; however, the highest rate of BJ meal most often produced the largest increases in biomass and N uptake, except for lettuce in year 2. The largest concentrations of extractable NH<sub>4</sub><sup>+</sup>-N (170 mg kg<sup>-1</sup> soil) and NO<sub>3</sub><sup>-</sup>-N (130 mg kg<sup>-1</sup> soil) were also measured in year 2 plots amended with the highest rate of BJ meal. Greater moisture in year 2 increased the release of allelochemicals from BJ meal, which inhibited germination and stimulated N mineralization of soil organic N. Laboratory incubations conducted with the same soils and meals indicated that 52 to 112% of the N added in the meals was mineralized in 21 d and that nitrification was inhibited by both BJ and SA meals. In the first weed harvest of year one, the 3% treatments and 1% BJ treatment reduced weed biomass by 75 to 86% relative to that in the no-meal plots. By the second harvest the only significant difference was between 1% BN (0.06 g m<sup>-2</sup>) and 1% BJ (1.07 g m<sup>-2</sup>). For the second year, first harvest, a significant treatment/species interaction was detected and the data were analyzed by species. Pigweed (*Amaranthus retroflexus*) biomass in 3% BJ plots was reduced by 74% relative to the no-meal treatment. This treatment also reduced chickweed (*Stellaria media*) biomass by 99% relative to the 1% meals. While pigweed biomass was reduced by 3% BJ in the early part of the season, by the second harvest this same treatment had the greatest pigweed biomass perhaps due to increases in plant available N. Results from a growth chamber experiment indicate that SA-amended soils resulted in a minimum of 75% reduction in emergence if lettuce seeds were planted earlier than 5 weeks after meal application. Brassicaceae meals have the potential to improve crop yields by increasing plant available N and reducing early season weed biomass, but that the effects are largely dependent upon climatic conditions.

## Introduction

Providing adequate levels of plant available N to organically grown crops is difficult without the use of high analysis, inorganic fertilizers (Eneji et al., 2002; Poudel et al., 2002). Many of the current methods of nitrogen management in organic farming systems do not supply adequate plant-available N, therefore limiting crop yield and production. Reduced yields due to N deficiency compromise the long-term sustainability of organic systems (Clark et al., 1999).

Typical methods of increasing plant-available N in organic systems include applications of composted manure and the planting of cover crops (Clark et al., 1999). A significant cause of concern with animal manure is the possibility of introducing pesticides and weed seeds. Additionally, complex chemical composition and immobilization of N by the microbial community as a result of high C:N ratios (Clark et al., 1999) lead to slow release of plant-available N from organic amendments. As a result, manure and cover crops may not increase N in time to meet the nutritional requirements of the growing crop, and crop yields suffer (Poudel et al., 2002). In addition to inadequate fertility levels, current weed control methods in organic systems are also limited. Direct physical control methods such as plastic or green mulching, harrowing, hoeing, and flaming have short-term effectiveness and can actually stimulate weed emergence (Barberi, 2001). These methods are also labor intensive and can be destructive to soil aggregation and structure (Ding et al., 2002). Thermal weed control is effective for many applications of surface weed eradication, but depth control is limited and hard-seeded annual weeds may not be effected (Bond and Grundy, 2001).

When oilseed crops in the Brassicaceae family such as rapeseed, canola, and mustard are crushed to release oil for processing, a byproduct known as seed meal is produced. Brassicaceae meals have a N content between 5 and 6 % and thus, may be suitable soil amendments in organic systems where higher analysis fertilizers are not available. The use of meal as a soil amendment is especially applicable in climates where cover-cropping is not possible. In addition to their N fertilizer benefits Brassicaceae seed meals contain glucosinolates, compounds that produce allelochemicals active against a variety of soil-borne plant pests. Glucosinolates are organic anions with specific side chains that distinguish one glucosinolate from another (Brown and Morra, 1997; Fahey et al., 2001). Members of the Brassicaceae have been shown to exhibit allelopathic properties as a result of glucosinolate-hydrolysis products such as isothiocyanates

(ITC), ionic thiocyanate ( $\text{SCN}^-$ ), nitriles, and oxazolidinethiones (OZT) (Vaughn, 1999; Chew, 1988; Sorensen, 1990), with isothiocyanates being considered the most biologically active of these glucosinolate hydrolysis products (Borek et al., 1994; Bending and Lincoln, 1999; Morra and Kirkegaard, 2002). Although the persistence of allelochemicals in soil is a concern that has led to questions over the acceptability of this application under organic standards (Stopes and Millington, 1991), the USDA has accepted allelopathy as a viable biocontrol method for use in organic farming systems (National Organic Standards, 1990).

Allelochemicals produced from Brassicaceae meals are general biocides and as such, may not only impact soil-borne plant pests, but other soil organisms as well. Although allelopathic effects of glucosinolate hydrolysis products from seed meal have been well documented (Brown et al., 1991; Ascard and Jonasson, 1991; Oleszek et al., 1994; Brown and Morra, 1995), very little is known about how these chemicals influence N mineralization and nitrification. Kirkegaard et al. (1999) found that soil concentrations of inorganic N were higher following Brassicaceae crops than legume crops and Bending and Lincoln (1999) found that synthetic ITCs inhibited nitrification in soil. Rates of mineralization and nitrification in Brassicaceae meal-amended soils, however, remain unknown.

Suppression of plant emergence by ITCs has been documented. 2-Propenyl, the dominant glucosinolate found in BJ tissue, was found to be as effective as the commercial soil fumigant methyl isothiocyanate with several crop and weed species (Vaughn and Boydston, 1997). Field research in Sweden (Johansson, 1992) suggested that SA can suppress weed growth and a greenhouse study by Ju et al. (1983) showed that ionic thiocyanate ( $\text{SCN}^-$ ), released by SA, inhibited the growth of tobacco and bean. Spiny sowthistle [*Sonchus asper* (L.) Hill], scentless mayweed (*Matricaria inodora* L.), barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.], and blackgrass (*Alopecurus myosuroides* Huds.) have all been determined to be susceptible to the ITCs produced by BJ and BN mulches in a greenhouse study (Petersen et al., 2001). Isothiocyanates are capable of inhibiting germination while seeds are still in the dormant stage (Vaughn and Boydston, 1995) suggesting that they can not only inhibit growth, but also the emergence of weeds.

A limitation of the use of the Brassicaceae seed meal involves possible inhibition of crop seed germination due to ITCs (Brown and Morra, 1996). In order for Brassicaceae meal to be a viable and feasible means of weed control in farming systems, planting dates that optimize weed

control but reduce the phytotoxic potential of the ITCs to the crop must be determined. While some literature does exist on the inhibitory effect of some plants in the Brassicaceae family on both weed and crop seed germination, little to no literature exists on suitable planting dates for crops that are sensitive to glucosinolate degradation products.

## Objectives

The original objectives of this project as stated in the proposal were to:

- 1) Test the effectiveness of high glucosinolate-containing meals at controlling soilborne diseases (damping-off and white mold of lettuce) in an organic farming system.
- 2) Determine the influence of these materials on N cycling processes in organic systems.

Visual assessments and measurements revealed negligible disease problems within the field during both years. Due to the lack of disease, the focus of our study was shifted to N cycling and weed control (not one of our original research objectives). To better understand meal N mineralization rates a third objective and laboratory study was added by the researchers. A fourth objective requested by OFRF following the review of our results from year one was to determine the suitable planting date for lettuce.

## Materials and Methods

### Meal Characterization

Defatted meal for glucosinolate characterization was produced using duplicate 1.1-g samples of seed ground with a Raney seed crusher and 5 ml of hexane (Raney et al., 1987). The glucosinolate concentration of each defatted meal was determined using a method similar to that of the International Organization of Standardization (1992). Briefly, glucosinolates were extracted by methanol, purified, enzymatically desulfated on ion-exchange resins, and quantified using reversed-phase high performance liquid chromatography (HPLC) with elution gradient and ultraviolet detection. To correct for different absorbance coefficients at 229 nm, detector absorbance values were multiplied by response factors currently determined by the international community to be the most accurate (Wathelet, personal communication). In order to determine specific seed meal effects on N and crop yield, three different Brassicaceae seed meals, *Sinapis alba* (SA), *Brassica juncea* (BJ), and *Brassica napus* (BN), were chosen based on their projected

glucosinolate profiles. *Sinapis alba* (variety IdaGold) has been shown to contain 4-OH-benzyl glucosinolate (Minchinton et al., 1982) and *Brassica juncea* (variety Pacific Gold) has been shown to contain 2-propenyl glucosinolate (Hanley et al., 1983). *Brassica napus* canola varieties Athena and Sunrise have low glucosinolate concentrations and were included as control meals expected to produce minimal amounts of glucosinolate-derived allelochemicals, while producing similar amounts of mineralized N. Sunrise was used the second year due to the lack of availability of Athena seed meal used during year one. Total C and N contents of the meals were determined by dry combustion (Nelson and Sommers, 1996) using a C/N/S analyzer (Elementar, Hanau, Germany).

### Field Study

A field study was developed on a certified organic farm in the Palouse region of northern Idaho. The soil was classified as Driscoll-Larkin silt loam, a Mollisol association (Soil Survey Staff, 1981). Prior to the study, the field was treated and cropped uniformly thus reducing variability in residual N. The year previous to our study, the field was fallowed. Plots were re-located to a new site within the same field the second year to eliminate any residual effects of meal application. The plots used during the second year were fallowed during the first year of the field study. The initial extractable inorganic N content of the field ranged between 4.8 and 9.7 mg kg<sup>-1</sup> soil in year one and 11.1-16.7 mg kg<sup>-1</sup> soil in year two. The experimental design was a split plot randomized complete block with meal application as the main plot and two crops as the split plot. There were six replicate blocks. Individual plots were 1.5 m<sup>2</sup> with 0.3-m borders between plots and a 1.5-m border around the entire site. Treatments included 1% BN, 3% BN, 1% BJ, 3% BJ, and no meal for year one. For year two, the treatments included 1% BN, 3% BN, 1% BJ, 3% BJ, 1% SA, 3% SA, and no meal. The rates of 1% and 3% (meal:air dry soil) were selected in an attempt to balance N production with effective weed control, and were based on a 3-cm depth of soil incorporation and measured soil bulk density values (Grossman and Reinsch, 2002). Although it varied slightly based on the total N content of each meal, amendment resulted in approximately 185 kg N ha<sup>-1</sup> (27 g N plot<sup>-1</sup>) for the 1% treatment and 554 kg N ha<sup>-1</sup> (82 g N plot<sup>-1</sup>) for the 3% treatment being added to each plot. Irrigation, in the form of overhead sprinklers was used as necessary.

Two crops, lettuce (*Lactuca sativa*) and beets (*Beta vulgaris*), were chosen to determine N uptake and yield in meal-amended plots. Plots were seeded approximately 14 d after meal application during the first year. Phytotoxicity appeared to limit lettuce germination and all plots were replanted 14 d later. Plots were planted 28 d after meal application the second year. Seeding rates were determined using a seed spacing of 0.64 cm for both beets and lettuce. Three 1.2-m rows were planted in each plot and neither beets nor lettuce were thinned. Seeds were all obtained from the same source and the same lot was used within a year.

Three soil core samples were obtained from each plot both prior to meal application and post harvest to determine changes in inorganic N within each treatment. The cores were split into four depths, 0-5, 5-10, 10-20, and 20-30 cm. Like depth increments for the three sub-samples were combined to form one representative sample for each depth and plot. Samples were air-dried, gently ground, and sieved to obtain the less than 2-mm fraction. Plant-available N ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) was determined by extraction with 2M KCl (Mulvaney, 1996). Concentrations of  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N (year 2 only), and  $\text{NH}_4^+$ -N in extracts were measured using an automated colorimetric procedure (Lachat, Milwaukee, WI).

Stand counts were taken once each year by counting emergence in the center row (1.2 m) of each plot. Weed biomass was measured by species in each plot twice a year for both years by clipping the plants at the soil surface. Weed biomass was oven dried at 60°C for two days and weighed. Yield data were obtained prior to the first fall frost by collecting plant biomass from the entire middle row within each plot. This sample was then used to estimate the biomass for the plot area. For beets, both tops and roots were collected. For lettuce, the above ground biomass was removed at the soil surface. Samples were dried at 60° C and weighed. Total N was determined on a ground sub-sample of biomass from within each plot using dry combustion (Nelson and Sommers, 1996) performed with a C/N/S analyzer (Elementar, Hanau, Germany). Total N uptake by the crop was determined by multiplying biomass weight by the percent N content.

#### Laboratory/Growth Chamber Studies

A laboratory experiment arranged in a randomized complete block design with five replicates split by day was performed to determine mineralization characteristics of Brassicaceae-amended soils. Twenty grams of Palouse silt loam were added to individual

incubation jars. Seven treatments were applied: 1 and 3 % (meal wt:soil wt) each of BN, BJ, SA, and no meal. Soils were kept moist at 60% water-filled pore space. Replicate jars were destructively sampled and the entire volume of soil was analyzed on days 0, 1, 3, 7, 21, and 28 for  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, and  $\text{NH}_4^+$ -N using the method described above for soil inorganic N characterization.

A high glucosinolate meal (SA), low glucosinolate meal (BN), and no-meal treatment were used to determine optimal planting dates for lettuce. Ten replicate 10.2 cm x 10.2 cm pots per treatment were filled with soil collected from the field site and amended with either 3% BN, 3% SA, or no meal. The experiment was carried out twice and maintained in controlled growth chambers. The humidity was set at 60%, day temperature at 24° C, night temperature at 21° C, and a 14-hour photoperiod was used. Pots were set up every week for 6 weeks so a staggered succession of meal application dates occurred. Pots were watered once a day with an even spray. Pots were planted simultaneously with 16 lettuce seeds in each, one week after the last meal application. All seeds were from the same lot and source. Emergence was determined by counting plants at 3, 7, 10, and 14 d after planting.

All data sets were statistically analyzed using SAS (release 8.02). PROC-GLM was used to determine significant differences in the average inorganic N, biomass, total plant N data, field stand counts of beets and lettuce, weed biomass, and plant emergence in the greenhouse study. If no crop-treatment interaction was found, data were not separated by crop. Biomass and total plant N were analyzed by crop due to a significant crop effect. Statistical differences in inorganic N were determined on log-transformed data. Statistical differences for laboratory mineralization data were determined on pooled ANOVA log-transformed data. Emergence data from the growth chamber study was transformed by using an arcsine square root transformation. If a significant interaction between weed species and treatment was not found, all species were combined.

## Project Results

### Meal characterization

As expected, glucosinolates in the meals varied in type and concentration (Table 1). The two *B. napus* meals selected as low glucosinolate control meals contained the lowest total glucosinolate concentrations, ranging from 20.1  $\mu\text{mol g}^{-1}$  in Athena to 17.2  $\mu\text{mol g}^{-1}$  in Sunrise.

Total glucosinolate concentrations in the mustard meals substantially exceeded those in the *B. napus* control meals with *S. alba* meal containing 165.8  $\mu\text{mol g}^{-1}$  and *B. juncea* meal 126.1  $\mu\text{mol g}^{-1}$ . The main difference between the two mustard meals was that 4-OH benzyl was the dominant glucosinolate in *S. alba* meal (148.1  $\mu\text{mol g}^{-1}$ ), whereas 2-propenyl was the primary glucosinolate in *B. juncea* meal (133.8  $\mu\text{mol g}^{-1}$ ). Total N content of the BN meals (5.2% for Athena and 5.3% for Sunrise) was similar to the other meals (5.8% for SA, and 5.6% for BJ), thus confirming the acceptability of using BN meal as a low glucosinolate N source to which high glucosinolate mustard meals can be compared.

Table 1. Glucosinolate concentrations for each meal studied.

Glucosinolate R-group (response factor)	<i>B. napus</i>	<i>B. napus</i>	<i>S. alba</i>	<i>B. juncea</i>
	“Athena”	“Sunrise”	“Ida Gold”	“Pacific Gold”
	----- $\mu\text{mol g}^{-1}$ of sample-----			
(2R)-2-hydroxy-3-butenyl (1.09)	1.5	1.3	3.4	0.5
2-propenyl (1.00)				123.8
(2S)-2-hydroxy-3-butenyl (1.09)	0.4			
2-hydroxy-4-butenyl (1.09)	0.2		1.8	
(2R)-2-hydroxy-4-pentenyl (1.09)				0.5
4-hydroxy-benzyl (0.28)			148.1	
Unknown (1.00)			9.1	
3-butenyl (1.11)	2.8	2.7		
4-hydroxy-3-indolylmethyl (0.28)	11.3	10.9		0.74
unknown (1.00)			2.6	
unknown (1.00)			0.74	
4-pentenyl (1.11)	1.3	1.4		
3-indolylmethyl (0.29)	0.9	0.8		
4-methylthiobutyl (1.00)	1.7			
N-methoxy-3-indolylmethyl (0.29)		0.1	0.01	0.6
unknown (1.00)				1.33
TOTAL	20.1	17.2	165.75	126.14

Field study

Crop yields and N uptake

For the first year, mean lettuce above ground biomass values in 3% BJ-and BN-amended soils (36.0 and 28.0 g m<sup>-2</sup>, respectively) were significantly higher and more than double that of the other treatments (Table 2). The average content of N in lettuce tissues was significantly higher in both BN treatments (3.9 % N) and 3% BJ (3.8 % N) than in 1% BJ (2.9 % N) and the treatment without meal (3.1% N) (Table 2).

Lettuce biomass in the second year was much greater than the first year and treatment differences differed from those observed in year one (Table 2). In contrast to year one, no meal-amended treatment yielded better than the no meal control. Ironically, the 3% BJ treatment that showed highest yields in year one, was the only meal-amended treatment in the second year to produce lower lettuce biomass (128.0 g m<sup>-2</sup>) than the control (323.6 g m<sup>-2</sup>). In the second year there were no significant differences in the percent N content of lettuce tissues among treatments (Table 2). Due to the low yield within 3% BJ plots, 1% SA, 3% BN and the no meal control all had significantly higher total plant N (Table 2).

Table 2. Lettuce yield and N uptake within plots for years one and two. Significant differences are indicated by different letters within a column for a specific year.

Treatment	Dry weight (g m <sup>-2</sup> )	N plant (%)	Total plant N (g m <sup>-2</sup> )
<u>Year 1</u>			
1% BN	11.2 b	3.9 a	0.4 a
3% BN	28.0 a	3.9 a	1.0 a
1% BJ	6.8 b	2.9 bc	0.2 a
3% BJ	36.0 a	3.8 a	1.2 a
No meal	10.2 b	3.1 bc	0.4 a
<u>Year 2</u>			
1% BN	260.8 ab	3.4a	9.6 ab
3% BN	337.4 a	3.9 a	13.8 a
1% BJ	307.2 ab	3.2 a	10.2 ab
3% BJ	128.0 b	4.0 a	5.4 b
1% SA	309.6 a	4.1 a	13.2 a
3% SA	331.0 a	3.5 a	10.2 ab
No meal	323.6 a	3.5 a	11.4 a

First year beet yields in all meal treatments except 3% BN (419 g) were significantly higher than in the no meal treatment (177.6 g) (Table 3). Total beet plant dry weights in the remaining meal-amended plots ranged from 458.6 g m<sup>-2</sup> for 1% BN to 698.0 g m<sup>-2</sup> for 3% BJ.

Only the 3% BJ treatment showed higher N concentration within the green portion of the plant than the control, whereas N concentrations in the roots of all meal-amended treatments were higher than the control. The total amount of N taken up by beets (roots + greens) was significantly higher in the 3% BJ-amended plots ( $23.2 \text{ g m}^{-2}$ ) than in all other plots for year 1 (Table 3). Other meal-amended treatments showed greater N uptake than the no meal control ( $4.2 \text{ g m}^{-2}$ ), but total plant N ranged from only 12.2 to  $14.2 \text{ g m}^{-2}$ .

Although in year two mean dry weights of beet greens and root biomass in 3% BJ-amended ( $6486.0 \text{ g}$ ) soils were more than twice those compared to other treatments, high variability in replicate biomass measurements nullified any ability to separate mean differences (Table 3). The N contents of beet greens ranged from 3% in no meal to 4.2 in 3% BJ plots and were significantly higher in all treatments compared to the no meal control (Table 3). All meals except 3% BN and 1% BJ significantly increased the N content of the roots relative to the no meal control (Table 3). Unlike the first year, 3% BJ was the only treatment in the second year that significantly increased total plant N uptake ( $158.0 \text{ g N}$ ) relative to the no meal control ( $55.2 \text{ g}$ ) in beet tissues (Table 3).

Table 3. Beet yield and N uptake within plots for years 1 and 2. Significant differences are indicated by different letters within a column for a specific year.

Treatment	Dry weight ( $\text{g m}^{-2}$ )	N greens (%)	N roots (%)	Total plant N ( $\text{g m}^{-2}$ )
<u>Year 1</u>				
1% BN	458.6 ab	3.2 b	2.0 b	12.2 b
3% BN	419.0 bc	3.4 ab	2.0 b	12.2 b
1% BJ	548.0 ab	3.1 b	2.0 b	14.2 b
3% BJ	698.0 a	3.7 a	2.8 a	23.2 a
No meal	177.6 c	3.0 b	1.5 c	4.2 c
<u>Year 2</u>				
1% BN	2773.6 a	3.7 ab	2.0 bc	66.0 ab
3% BN	2557.4 a	3.9 ab	1.8 cd	57.8 ab
1% BJ	3119.8 a	3.7 ab	1.9 cd	65.4 ab
3% BJ	6486.8 a	4.2 a	2.5 a	158.0 a
1% SA	3139.2 a	3.5 b	2.3 abc	78.6 ab
3% SA	2855.8 a	3.9 ab	2.1 bc	72.0 ab
No meal	2763.8 a	3.0 c	1.7 d	55.2 b

## Nitrogen mineralization

No significant crop-treatment interactions were detected so field N mineralization data from both lettuce and beets were combined for both years 1 and 2. Significant differences in inorganic nitrogen, based on pre-meal application and post-harvest soil samples, were not detected in the 10 to 15- or the 15 to 20-cm depths during each year. Therefore, only data from the 0 to 5- and 5 to 10-cm depths are shown. In year one at the 0 to 5-cm depth, all meal-amended treatments produced similar amounts of  $\text{NO}_3^-$ -N (90.8 to 141.0  $\text{mg kg}^{-1}$  soil) and all showed higher  $\text{NO}_3^-$ -N concentrations than the no meal control (17.7  $\text{mg kg}^{-1}$  soil) (Figure 1a). In contrast to  $\text{NO}_3^-$ -N, only  $\text{NH}_4^+$ -N concentrations in the 3% BJ treatment (37.9  $\text{mg kg}^{-1}$  soil) exceeded that of the control (3.8  $\text{mg kg}^{-1}$ ). Significant differences in residual  $\text{NH}_4^+$ -N or  $\text{NO}_3^-$ -N between the meal treatments and the no meal treatments were not detected at the 5 to 10-cm depth for year one (Figure 1b).

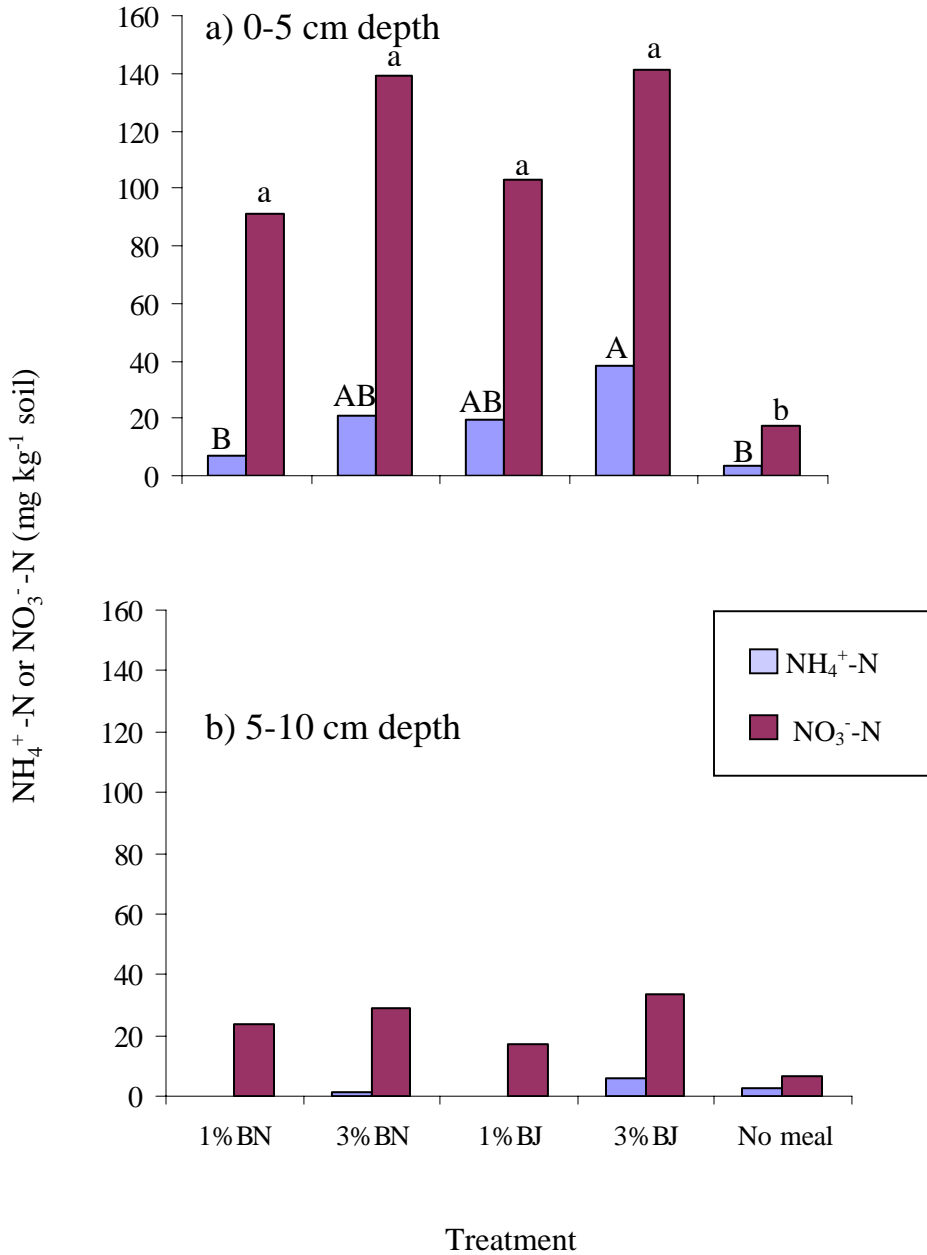


Figure 1. Change in inorganic N (post harvest-pre meal application) during year 1 in meal-amended plots planted to beets and lettuce at a) 0 to 5 cm and b) 5 to 10 cm depths. Different upper case letters indicate statistical differences ( $\alpha=0.05$ ) in NH<sub>4</sub><sup>+</sup>-N among treatments. Different lower case letters indicate statistical differences in NO<sub>3</sub><sup>-</sup>-N. Lack of a horizontal bar indicates no net change in NH<sub>4</sub><sup>+</sup>-N or NO<sub>3</sub><sup>-</sup>-N. There were no significant differences among treatments below the 0 to 5 cm depth.

For year two, within the 0 to 5-cm depth, there was a trend for increased soil N in the meal-amended treatments as compared to the control, but only the 3% BJ treatment produced greater  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations than the no meal control (Figure 2a). Changes in ammonium were higher in the 3% BJ treatment ( $167.6 \text{ mg NH}_4^+\text{-N kg}^{-1}$  soil) than all treatments except 3% BN ( $102.4 \text{ mg NH}_4^+\text{-N kg}^{-1}$  soil) (Figure 2a). The 3% BJ treatment also produced the greatest residual  $\text{NO}_3^-$ -N ( $125.3 \text{ mg kg}^{-1}$  soil) compared to that found in the remainder of the treatments and the control (Figure 2a). For the 5 to 10-cm depth, 3% BJ produced greater residual  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations than all other treatments and the control except for a similar  $\text{NO}_3^-$ -N concentration measured in the 3% BN treatment (Figure 2b).

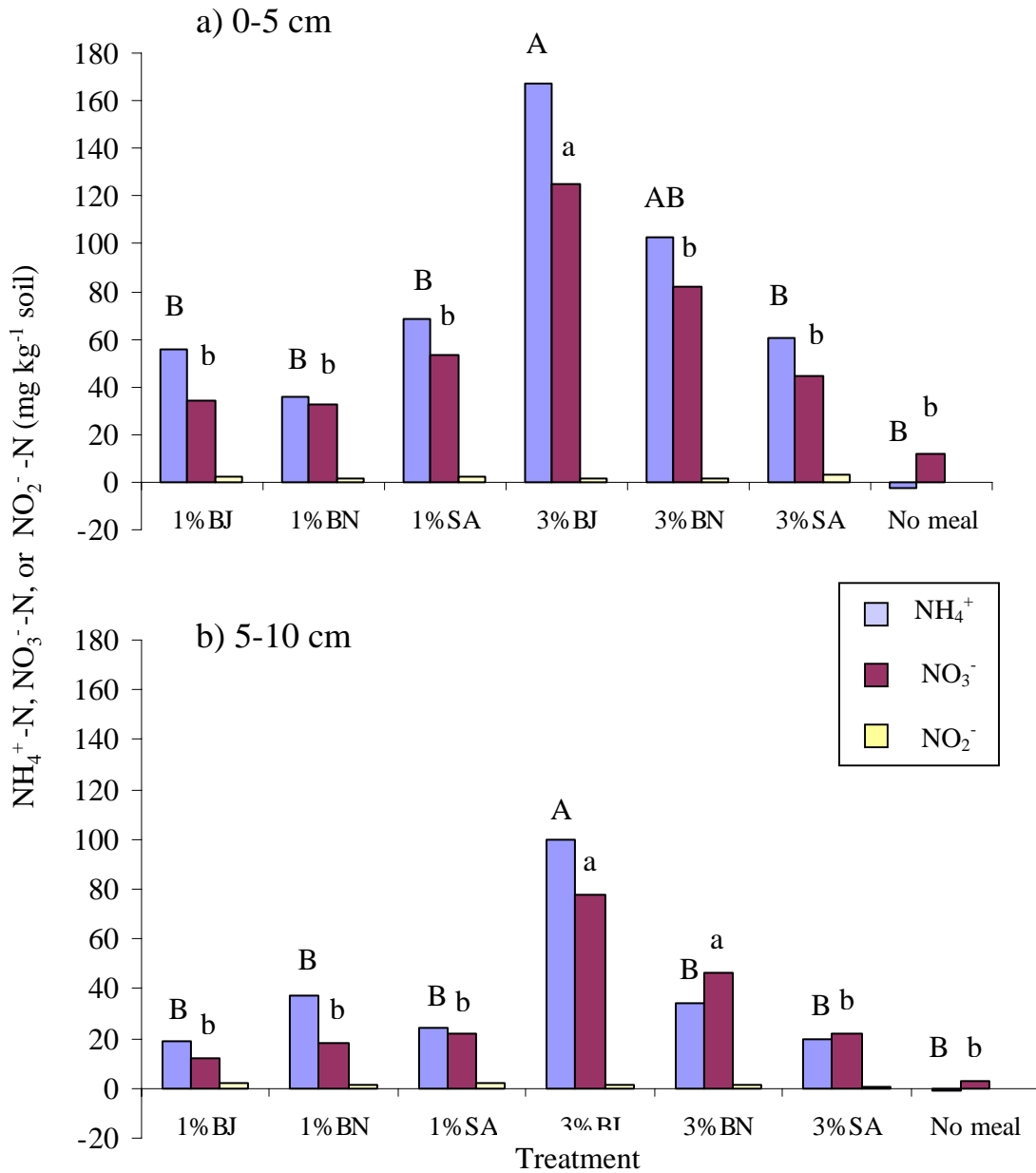


Figure 2. Change in inorganic N (post harvest-pre meal application) during year 2 in meal-amended plots planted to beets and lettuce at a) 0 to 5 cm and b) 5 to 10 cm depths. Different upper case letters indicate statistical differences ( $\alpha=0.05$ ) in NH<sub>4</sub><sup>+</sup>-N among treatments. Different lower case letters indicate statistical differences in NO<sub>3</sub><sup>-</sup>-N. Lack of a horizontal bar indicates no net change in NH<sub>4</sub><sup>+</sup>-N or NO<sub>3</sub><sup>-</sup>-N. There were no significant differences among treatments below the 5 to 10 cm depth so the data are not shown.

## Weeds

The results presented for year one are a combination of all weed species present due to the lack of a significant species-treatment interaction. For year one, harvest one, average weed biomass ranged from 1.85 g m<sup>-2</sup> in the no meal treatment to 0.24 g m<sup>-2</sup> in the 3% BN treatment. All meal treatments except 1% BN resulted in significantly lower weed biomass compared to the no meal treatment (Figure 3). Weed biomass for the second harvest of year one ranged from 1.06 g m<sup>-2</sup> in the 1% BJ treatment to 0.06 g m<sup>-2</sup> in the 1% BN treatment with the only significant differences being between these two treatments (Figure 4). Weed biomass in plots amended with 3% BN was not different from that in 3% BJ for either of the harvests during year one. Weed biomass in the 3% BN treatment was lower than the no meal treatment for the first harvest but the same two treatments were not statistically different for the second harvest.

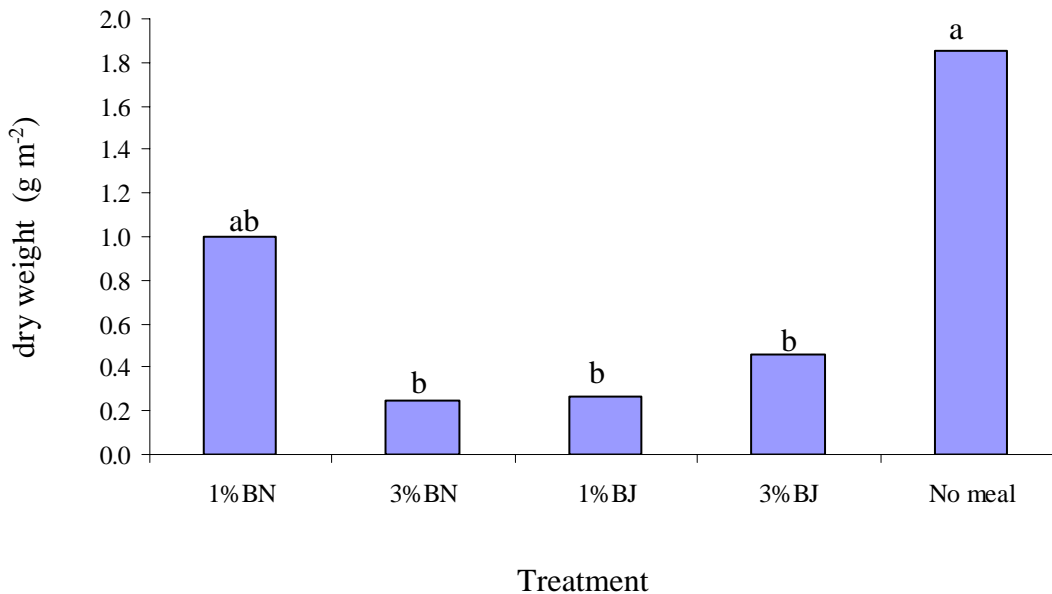


Figure 3. First biomass harvest, year one. Due to the limited distribution of species, data are shown as the average for all weed species combined. Differing letters indicate statistical differences among treatments.

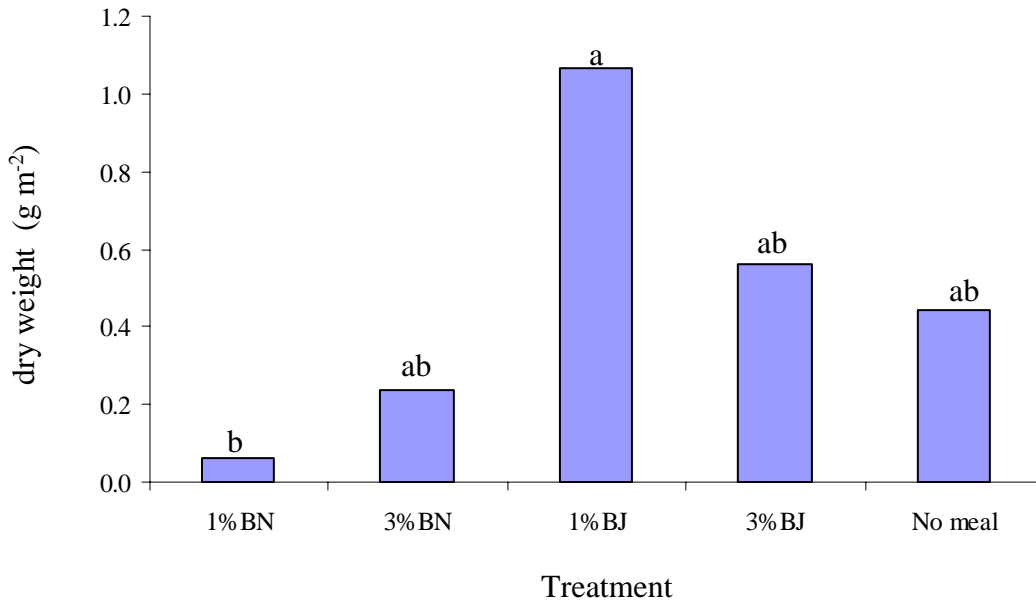


Figure 4. Second biomass harvest of weeds, year one. Significant treatment-species interactions did not exist so the data were combined by species. Differing letters indicate statistical differences among treatments.

For year two, there was a significant species-treatment interaction for pig weed and chickweed, the two dominant weed species present. The 3% BJ treatment inhibited chickweed biomass by approximately 99% relative to the 1% meals, but did not inhibit chickweed biomass relative to the other 3% meal treatments or to the no meal treatment (Figure 5). Three percent BJ was the only treatment that significantly reduced (74% reduction) pigweed biomass compared to the no meal treatment for the second year first weed biomass harvest. Average pigweed biomass was highest in the second harvest of year two in the 3% BJ treatment (39.3 g m<sup>-2</sup>) relative to all other treatments (Figure 6).

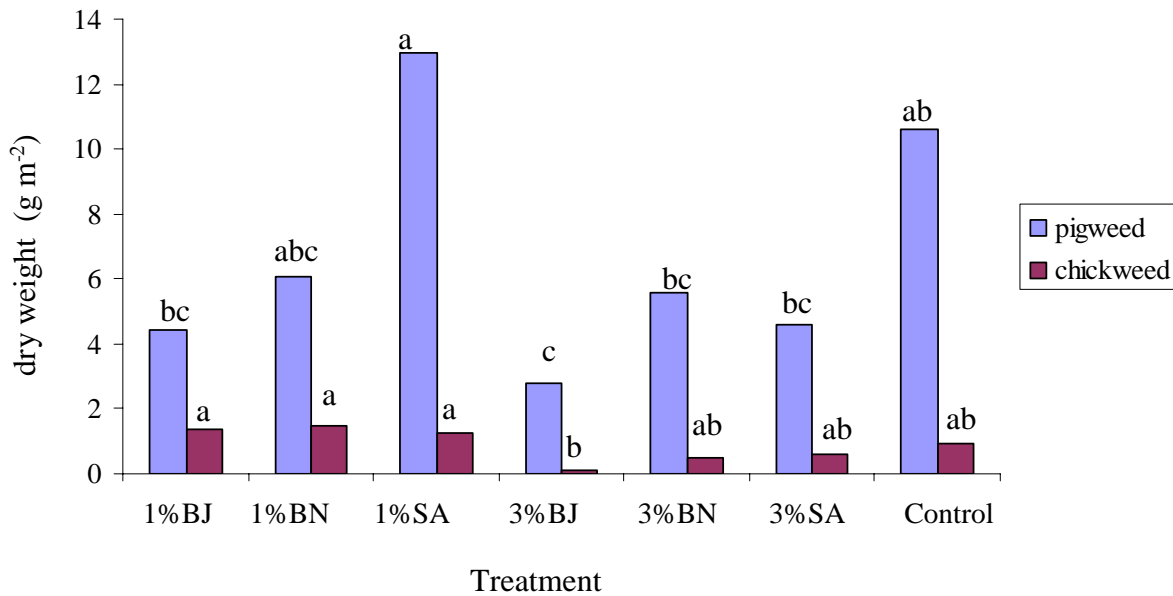


Figure 5. First biomass harvest of all weed species, year two. Percent species composition is shown in Table 2. Differing letters indicate statistical differences among treatments within a weed species.

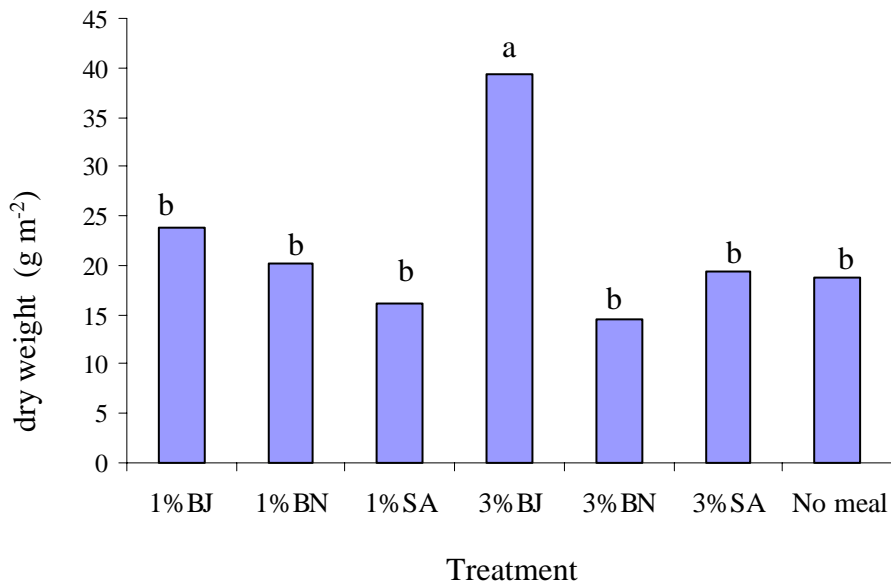


Figure 6. Pigweed biomass in the second harvest, year two. Differing letters indicate statistical differences among treatments.

### Lettuce and beet emergence

There were no significant interactions between crop and treatment for stand counts (crop emergence) in the field study for either year, thus the data are presented as averages for the two crops. For the first year, average stand counts for the crops ranged from 39.0 plants per 1.2 m row in the 1% BJ and 3% BN treatments to 23.5 plants per 1.2 m row in the no meal treatment (Figure 7). Three percent BN (39.3 plants per 1.2 m row) and 1% BJ (39.4 plants per 1.2 m row) resulted in significantly higher stand counts compared to the no meal treatment (23.7 plants per 1.2 m row). Unlike data from year one, emergence in the no meal treatment was significantly higher (80.5 seeds per 1.2 m row) than all other treatments for the second year stand count (Figure 8).

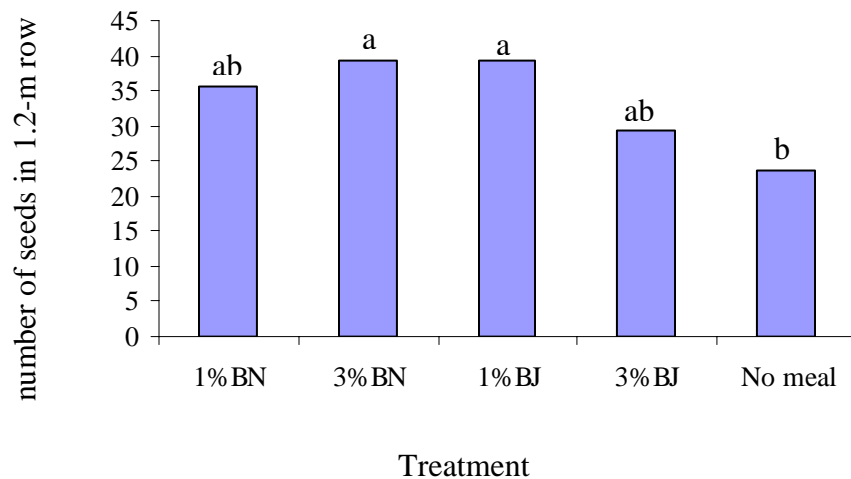


Figure 7. Crop stand counts for year one of the field study. Different letters indicate statistical differences among treatments.

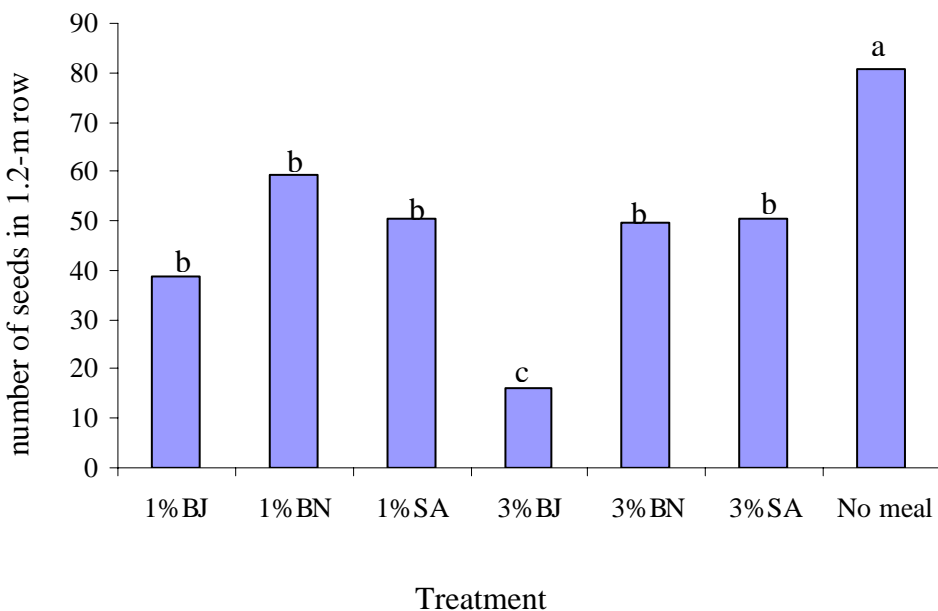


Figure 8. Crop stand counts for year two of the field study. Different letters indicate statistically significant differences among treatments.

#### Laboratory meal mineralization study

Significant differences in N mineralization among all meal varieties first occurred on day 3 (Table 4). The lowest concentration of inorganic N on day 3 was produced by the 3% BJ (39.7 mg N kg<sup>-1</sup> soil) treatment, an inorganic N concentration even lower than that of the no meal control (65.8 mg N kg<sup>-1</sup> soil) (Table 4). Highest inorganic N concentrations at day 3 were produced in 1 (171.0 mg N kg<sup>-1</sup> soil) and 3% (425.3 mg N kg<sup>-1</sup> soil) BN treatments. At day 7 and beyond, statistical analyses indicated that in most cases the 3% meal treatments produced greater amounts of inorganic N than the 1% treatments. All meal-amended treatments produced greater amounts of inorganic N than the no meal control from day 7 to day 28. Trends showed that inorganic N increased in each respective treatment in days 3 to 21 after which on day 28, inorganic N in all meal-amended treatments except 3% SA decreased. As a result, the 3% SA treatment produced the highest amount of inorganic N (1505.6 mg kg<sup>-1</sup> soil) at the end of the 28-d period.

Table 4. Mean values of inorganic N ( $\text{mg N kg}^{-1}$  soil) present on day zero and mineralized on days 1 to 28 as determined in the laboratory incubation experiment. Significant differences of treatments for any one specific sampling time are indicated by different letters within a column.

	Sampling time (d)					
	0	1	3	7	21	28
1%BJ	26.5 a	21.2 a	76.8 d	369.8 b	663.3 b	248.0 c
1%BN	26.3 a	20.8 a	171.0 b	379.8 b	450.5 b	367.4 c
1%SA	29.7 a	31.9 a	76.4 d	233.8 b	597.5 b	434.7bc
3%BJ	32.1 a	41.7 a	39.7 f	542.6 ab	1300.5 a	597.9 b
3%BN	30.1 a	18.1 a	425.3 a	698.9 a	1252.6 a	706.6 b
3%SA	37.2 a	44.5 a	73.4 c	614.9 a	981.2 ab	1505.6 a
No meal	25.4 a	32.4 a	65.8 e	74.7 c	41.8 c	30.5 d

Ammonium was the dominant inorganic N species present on all days of the incubation (Figure 9 a, b, and c), and thus this N species was responsible for most of the trends in total N as described above. Only  $\text{NH}_4^+$ -N was detected until day 21 at which time the 3% BN treatment showed the highest  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N concentrations. Nitrite and  $\text{NO}_3^-$ -N concentrations at day 28 were significantly higher in 1 (24.6  $\text{mg NO}_2^-$ -N  $\text{kg}^{-1}$  soil and 42.4  $\text{mg NO}_3^-$ -N  $\text{kg}^{-1}$  soil) and 3% (18.9  $\text{mg NO}_2^-$ -N  $\text{kg}^{-1}$  soil and 46.7  $\text{mg NO}_3^-$ -N  $\text{kg}^{-1}$  soil) BN-amended treatments than all other treatments (Figure 9 b and c).

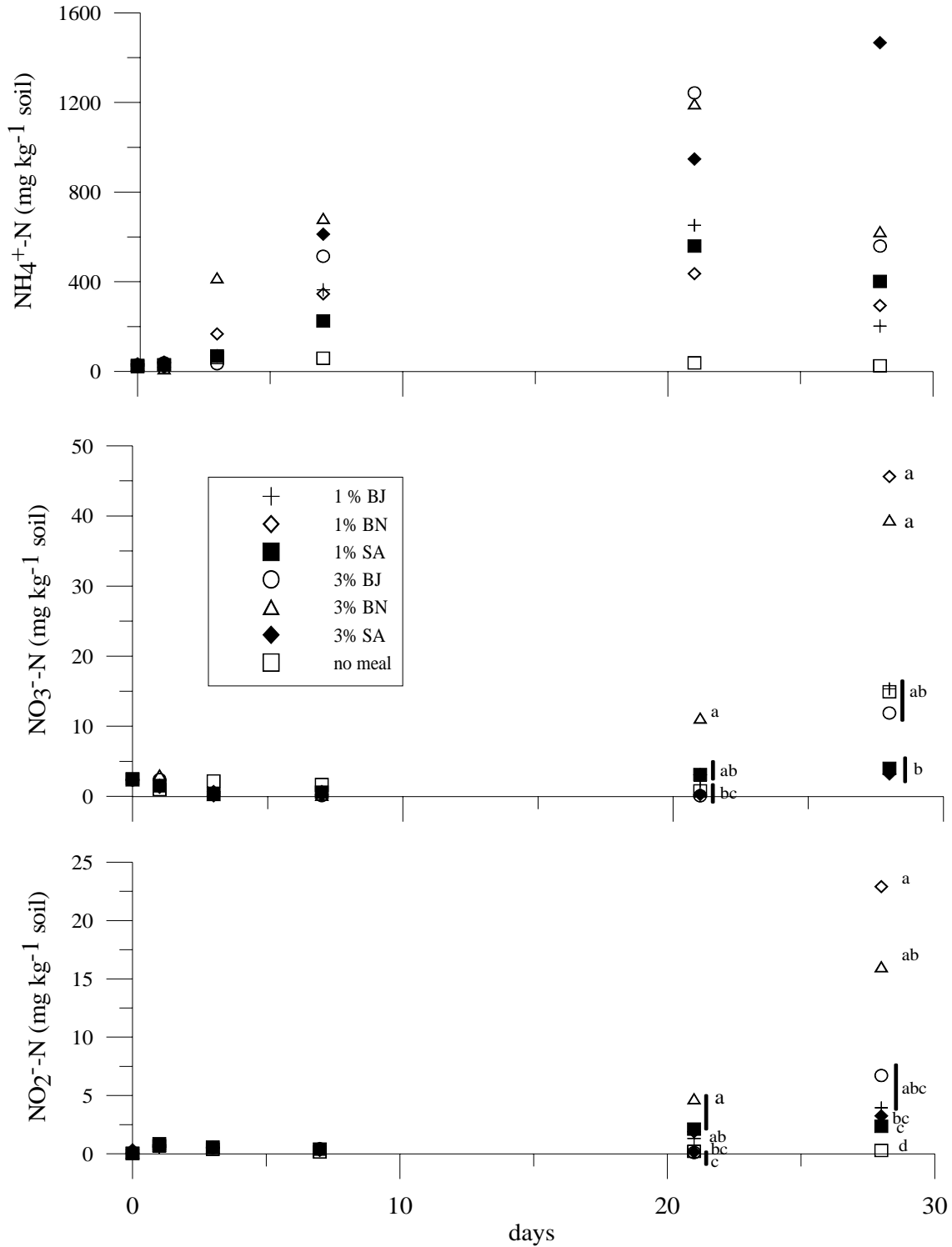


Figure 9. Extractable a)  $\text{NH}_4^+\text{-N}$ , b)  $\text{NO}_3^-\text{-N}$ , c)  $\text{NO}_2^-\text{-N}$  in  $\text{mg N kg}^{-1}$  soil measured in a 28-d laboratory incubation experiment. Significant differences for  $\text{NH}_4^+\text{-N}$  concentrations are identical to those shown in Table 4 for total inorganic N. Significant differences for  $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$ , for days 21 and 28, are indicated by different letters within a day.

### Growth chamber study

The results from the first and second growth chamber study showed the same statistical differences and thus data were combined. Lettuce emergence between the 7 and 14 day counts remained the same, with no significant die off or emergence occurring, thus only the 14-day count is presented. Emergence was significantly lower in SA-amended soil relative to the no-meal treatment from week one to week four. During this time, emergence in the SA-amended treatment ranged from 0.5 seeds (3% emergence) to 3.1 seeds (17% emergence) (Figure 10). Emergence in the BN treatments from 7.5 seeds (42%) in week one to 8.7 seeds (48%) in week four. Emergence within the BN treatment was only significantly different from that within the no-meal treatment during the second week of the experiment. For weeks five and six, emergence of lettuce seeds was not significantly different among the three treatments (Figure 10).

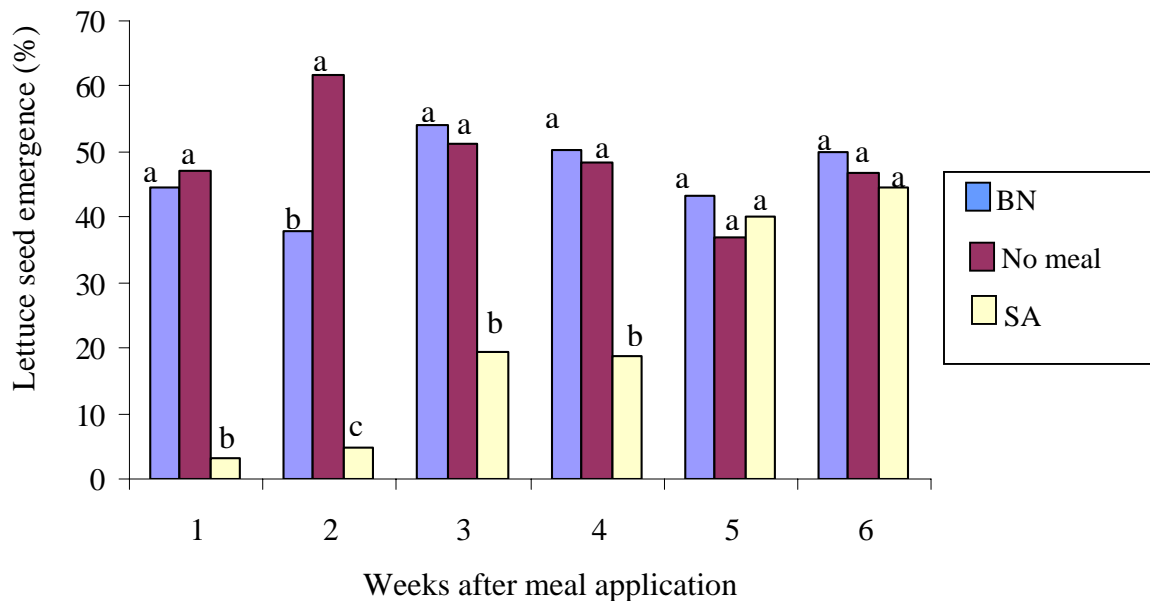


Figure 10. Emergence of lettuce seeds in 3% *B. napus* (BN), *S. alba* (SA), and no-meal amended pots two weeks after planting in the growth chamber. Within a week, different letters indicate statistical difference among treatments.

## Conclusions and Discussion

### Meal Characterization

Glucosinolate analyses demonstrated that the two BN meals contained low glucosinolate concentrations and N contents similar to the other meals (5.8% for SA, and 5.6% for BJ), thus confirming the acceptability of using these meals as low glucosinolate N sources to which high glucosinolate mustard meals can be compared. Specific glucosinolate profiles of the seed meals were consistent with data reported in the literature. *S. alba* meal was dominated by 4-OH benzyl glucosinolate (Table 1) that when hydrolyzed produces 4-OH-benzyl ITC, an unstable compound that very rapidly generates as one of its products  $\text{SCN}^-$  (Borek and Morra, in preparation). Thiocyanate is water soluble, primarily degraded by microbes, and less biologically active than ITCs (Brown and Morra, 1993). It is known to be phytotoxic, inhibiting plant growth and emergence (Beekhuis, 1975; Ju et al., 1983). In contrast, BJ meal contained high concentrations ( $123.8 \mu\text{mol g}^{-1}$ ) of 2-propenyl glucosinolate (Table 1) that produces the more stable and volatile 2-propenyl ITC (Vaughn, 1999). Increased volatility has been found to increase the toxicity of ITCs to microbial growth (Lewis and Papavizas, 1971; Kanemaru and Miyamoto, 1990), thus making 2-propenyl ITC potentially more toxic to microbes than the compounds produced by SA meal.

Similar to the findings of Minchinton et al. (1982), SA (variety Ida Gold) contained 4-OH-benzyl glucosinolate (Table 1) which can form  $\text{SCN}^-$  (Beekhuis, 1975). Thiocyanate is known to inhibit germination and seedling emergence (Beekhuis, 1975; Ju et al., 1983). Mechanisms behind the toxicity of  $\text{SCN}^-$  are unclear, but possibilities include, 1)  $\text{NH}_4\text{SCN}$  acting as a protoplasmic poison by coagulating proteins or combining with cell constituents (Harvey, 1931), 2) reduction of the activity of catalase by up to 65%, possibly due to  $\text{SCN}^-$ 's affinity for iron resulting in toxicity to the plant (Landen, 1934), and 3) inhibition of photosynthetic  $\text{O}_2$  release resulting in an inability to carry out the Hill reaction (Wu and Basler, 1969). Ju et al. (1983) found that increased chlorosis corresponded to increased  $\text{SCN}^-$  concentration in tissue, thus suggesting that the physiological effect of  $\text{SCN}^-$  is related to its ability to chelate iron, thereby reducing iron availability for chlorophyll synthesis.

*B. juncea*, (variety Pacific Gold) contained 2-propenyl glucosinolate (Table 1), consistent with the findings of Hanley et al. (1983). 2-Propenyl has been shown to be inhibitory to a number of organisms (Matthiessen and Shackleton, 2000). Mechanisms for toxicity of 2-propenyl ITC are unclear, although it has been proposed that seed germination inhibition is a result of the ability of ITCs to bind to proteins which results in inhibition of metabolic processes (Brown and Morra, 1997).

The two BN canola seed meal varieties (Athena and Sunrise) have low glucosinolate contents (Milford and Evans, 1991) compared to those found in the mustard meals. The dominant glucosinolate in both varieties was 4-OH-indolyl-3-methyl glucosinolate (Table 1) as indicated by Quinsac et al. (1991). Although 4-OH-indolyl-3-methyl is a non-ITC producing glucosinolate (Brown and Morra, 1996) it can potentially degrade to SCN<sup>-</sup>.

## Field Study

### Crop yields

Differences in lettuce and beet biomasses between years one and two make interpretation of meal-amendment effects on plant yields somewhat difficult. The higher biomass observed the second year as compared to the first (Tables 2 and 3) for both beets and lettuce was most likely a result of differing climatic conditions since initial plant-available soil N was similar for both years. During the first year the study area experienced a very dry spring, while the second year it experienced a very wet spring that caused more than a ten-fold increase in biomass. Despite biomass differences between years, trends in plant dry weights indicate that Brassicaceae meals can increase the yield of lettuce and beets compared to the no-meal control, but results are altered greatly by climatic conditions.

Data indicate that early season moisture differences influenced crops yields not only in a direct fashion by altering seed germination, but also indirectly by controlling the formation and dissipation of phytotoxic allelochemicals. This is best illustrated by noting that one of the highest lettuce yields was recorded for 3% BJ-amended plots in year one and one of the lowest yields for 3% BJ-amended plots in year two. Germination of small-seeded crops such as lettuce, thus appears especially susceptible to inhibition by glucosinolate-derived allelochemicals originating from mustard meals.

2-Propenyl glucosinolate in BJ meal is known to produce 2-propenyl ITC, a compound that evidently had a detrimental impact on lettuce yield in year two, but not in year one. Greater amounts of precipitation that occurred after meal application and prior to planting in year two, most likely caused more complete hydrolysis of glucosinolates contained within the meal, thus creating a flush of ITC that inhibited plant emergence. It is also probable that increased soil moisture extended ITC residence time possibly by inhibiting volatile losses (Borek et al., 1995). Without sufficient precipitation in year one, no such ITC flush and residence time increase occurred and lettuce yields were not reduced by 3% BJ meal. The larger-seeded beet crops showed no indication of germination inhibition by glucosinolate-derived allelochemicals, thus demonstrating that meal amendment rate recommendations must be made on a crop-specific basis.

Given the results observed with BJ meal, the lack of a lettuce yield response to SA meal at the highest amendment rate in year two is at first perplexing, especially since the expected product,  $\text{SCN}^-$ , has been shown to be phytotoxic (Beekhuis, 1975; Ju et al., 1983). In this case it is likely that any highly soluble  $\text{SCN}^-$  produced was leached out of the seed zone and moved below the depth at which it may have contacted the actively germinating seed. Results with both high glucosinolate mustard meals indicate that generalizations concerning a yield response require consideration of glucosinolate type present in the mustard meal, crop sensitivity to glucosinolate hydrolysis products, and potential variability in climatic conditions.

#### Nitrogen uptake by lettuce and beets

Increased N uptake is expected in amended plots since low C:N ratios of the meals (8.5:1 for BJ, 8.1:1 for SA and 9.1:1 for BN) inherently encourage net mineralization. Non-significant trends indicate that total lettuce N expressed on an area basis was highest in the 3% treatments (Table 2). In the second year there were no significant differences in the percent N content of lettuce tissues among treatments (Table 2). Total plant N expressed on an area basis was lowest in the 3% BJ treatment in the second year, because allelochemicals released by the meal inhibited seed germination and reduced overall crop yields.

For beets in year one, only the 3% BJ treatment showed a higher N concentration within the green portion of the plant relative to the no-meal control, whereas N concentrations in the roots of all meal-amended treatments were higher than the control. As a combined result of

yield and N uptake, amounts of total plant N per unit area were greater in all meal-amended treatments than the no meal control (Table 3). In year two, N contents of beet greens were significantly higher in all treatments compared to the no meal control (Table 3). Unlike the first year, 3% BJ was the only treatment in the second year that significantly increased total plant N uptake ( $158.0 \text{ g m}^{-2} \text{ N}$ ) relative to the no meal control ( $55.2 \text{ g m}^{-2} \text{ N}$ ) in beet tissues (Table 3). Unlike lettuce, there was no obvious negative effect of plant-derived allelochemicals on seed germination or N uptake by beets; however, as will be discussed below, meal-derived allelochemicals may play a role in N mineralization.

### N mineralization

Net N mineralized can be estimated by first calculating the difference between inorganic N measured pre-plant to that determined post harvest, and then adding this value to the amount of N present in the tissues of each respective crop within a treatment. Assuming that relatively consistent soil properties across the field lead to uniform N losses, these estimates yield values that range from  $4.2 \text{ g N m}^{-2}$  in the no-meal treatment planted to beets in year 1, to  $278 \text{ g N m}^{-2}$  for 3% BJ plots planted to beets in year 2 (Table 5). Net mineralized N across all meal treatments ranged from between 2 to 18 times that measured within the plots that didn't receive meal. The largest amount of mineralized N occurred within 3% BJ plots within each crop for each year. The amount of N mineralized in 3% BJ plots was 1.4 to 5 times greater than the actual amount of N added in the form of meal. Integrating residual soil inorganic N values with plant uptake clearly shows, therefore, that meal amendment resulted in increased N mineralization beyond that of the no-meal control and that not all organic N mineralized could have originated from the meal itself.

The largest amount of N mineralized consistently occurred across crop and year in the 3% BJ treatments, suggesting that specific compounds in this meal may stimulate mineralization of soil organic N. We propose that Brassicaceae seed meal application may indirectly influence N release through the impact of ITCs on soil microorganisms. This seems plausible given the fact that the largest impact was observed by 3% application of BJ meal, the meal that produces the highest concentration of the most biologically active ITC (2-propenyl). The differential effect of 3% BJ meal was most dramatic in year two (Fig. 2 and Table 5), because as argued with lettuce yields, increased moisture in year two resulted in more complete glucosinolate hydrolysis.

Nitrogen mineralization and hence residual soil N values measured in year 1 were not significantly impacted by ITC production and no differences were detected among the meal treatments (Fig. 1).

Table 5. The amount of N added as meal-N and net mineralized N (residual N + plant tissue N) in each treatment for both lettuce and beets in year one and two.

	N added with meal (g N m <sup>-2</sup> )	Net mineralized N*	
		Year 1	Year 2
		Lettuce	Beets
1% BN	17.6	86.5	23.1
3% BN	52.8	99.4	67.0
1% BJ	18.6	104.2	14.2
3% BJ	55.8	129.7	75.7
1% SA	-	-	-
3% SA	-	-	-
Nomeal	-	22.8	4.2
1% BN	18.1	54.0	105.5
3% BN	54.2	100.2	168.03
1% BJ	18.6	54.2	107.8
3% BJ	55.8	249.2	277.9
1% SA	19.7	98.1	126.9
3% SA	59.2	67.4	138.6
No meal	-	24.2	58.2

\* Net mineralized N is estimated by [Nplant tissue + (Npostharvest – Npreplant)]

Isothiocyanates may increase plant available N by 1) acting as general biocides killing a portion of the microbial biomass and creating a flush of N similar to what is seen after synthetic fumigant application, 2) reducing the activity of microbes responsible for immobilizing N, 3) stimulating appropriate species associated with N mineralization, 4) suppressing bacteria antagonistic to those responsible for mineralization, and 5) inhibiting nitrification such that the

potential for  $\text{NO}_3^-$  leaching is reduced (Kirkegaard et al., 1999; Bending and Lincoln, 2000). Additional insight into the responsible mechanisms can be gained by more closely examining changes in N species as they occur in different years and with different meal amendments.

The ratio of  $\text{NH}_4^+$ -N to  $\text{NO}_3^-$ -N as determined for soils of meal-amended plots differed substantially in years 1 and 2, implying that nitrification rates varied between the years. Nitrification in year one was more extensive with  $\text{NO}_3^-$ -N detected as the dominant species (Figure 1), whereas in year two  $\text{NH}_4^+$ -N was the dominant species and concentration differences between the two species were not as great (Figure 2). Nitrification is known to be inhibited by allelochemicals (Bremner and McCarty, 1993; Bending and Lincoln, 2000, Paul and Clark, 1996), however similar values among meal types with contrasting glucosinolates and concentrations suggest that climatic differences between the two years are responsible, not the production of allelochemicals.

Higher rainfall may have increased the  $\text{NH}_4^+$ -N to  $\text{NO}_3^-$ -N ratio in soils collected during year 2 through 1) preferential leaching of  $\text{NO}_3^-$  below the zone that was sampled (30 cm) or 2) inhibition of nitrification caused by high soil moisture and relatively low  $\text{O}_2$  concentrations. Unlike year 1 there is indeed evidence of increased inorganic N in soil samples obtained from the 5 to 10 cm depth, but because  $\text{NH}_4^+$  is still dominant it would appear that  $\text{NO}_3^-$  movement cannot be responsible for decreased nitrification in the 0- to 5-cm samples. This is supported by the lack of differences in soil  $\text{NO}_3^-$ -N from 10 cm to the maximum sampling depth of 30 cm.

A large proportion of the inorganic N produced most likely remained in the more immobile  $\text{NH}_4^+$ -N form because of decreased rates of nitrification. Wet environmental conditions, as occurred in the spring of year 2, have indeed been shown to inhibit nitrification (Paul and Clark, 1996; Coyne, 1999). What remains puzzling is the increased amount of mineralization that took place as a result of 3% BJ amendment and the fact that such increases in inorganic N were not only observed in soils from 0 to 5 cm, but also at depths from 5 to 10 cm, well below the depth of meal incorporation.

The combined field data indicate that allelochemicals produced in the 3% BJ meal-amended soil, most likely 2-propenyl ITC, increased mineralization of soil organic N. Although we cannot determine which of the previously listed mechanisms are responsible, there is no evidence to suggest that nitrification inhibition played a major role in our observed results. It appears that the responsible allelochemicals were distributed in the soil by moisture inputs,

thereby increasing N mineralization not only in the zone of meal incorporation, but also in soil as deep as 10 cm. To achieve such an impact, sufficient moisture is necessary to distribute the allelochemicals and promote glucosinolate hydrolysis.

## Weeds

The noticeable variability in weed biomass between years (Figures 3-6) may be a result of an unusually dry spring the first year and an unusually wet spring the second year. Plots were planted a month later during the second year due to the wet spring, which resulted in weed biomass harvest occurring one month later than it did in the first year. The difference in weed harvest dates between the years may have also contributed to variation in species composition and the higher biomass seen in the second year. In addition to different harvest dates, the plots were moved the second year to reduce residual effects from the meal applied to the plots in the first year of this study. As a result, the weed seed bank and thus the species composition may have changed. The change in plot locations and the later collection date from year one to year two introduced significant variability into the data and likely caused the dominance of pigweed in the second year (Figure 5 and 6). Significant weed species-treatment interactions were seen for the first biomass harvest but not for the second for both years.

Although there was a significant weed species-treatment interaction for the first weed biomass harvest during the first year, the rarity of species in replicates (several species only occurred in one of the five replicate plots within a treatment) did not allow for statistical analysis, at the individual species level for this collection date. All meal treatments except for 1% BN reduced total weed biomass from 87 % (3% BN) to 76% (3% BJ) relative to the no meal treatment (Figure 3). The second weed biomass harvest did not show significant weed species-treatment interaction, thus this data is shown as a combination of species (Figure 4). There were no seed-meal amendments that reduced weed biomass in the second harvest relative to the no meal treatment.

There was a significant weed species-treatment interaction for the second year first harvest, thus the species present were analyzed separately (Figure 5). Pigweed was the dominant species present making up between 65 and 97% of the total weed biomass across treatments with lesser amounts of chickweed (4 to 35% of total weed biomass). This may indicate that 1) pigweed inherently produces more biomass than chickweed, 2) pigweed was more competitive

than chickweed for water and nutrients including N mineralized from the meal, and/or 3) differences in weed seed bank resulted in the differences in biomass.

The first harvest of pigweed during the second year indicated 74% suppression by 3% BJ relative to the no meal treatment (Figure 5). By the second harvest, pigweed biomass within the 3% BJ treatment was 52% higher than it was in the no meal treatment (Figure 6). The rebound may have resulted from changes in the plant available N released from BJ meal. Pigweed is considered a nitrofile (Teyker et al., 1991) and appears to be able to capitalize on the rapid mineralization of plant available N from BJ. The pigweed data indicate that the ability of weed species to take up extra N released from the meal may result in higher weed biomass later in the season after the allelopathic effect has worn off. The ability to promote late season weeds may limit the use of meal or require repeated applications over the growing season.

Significant weed suppression was not evidenced in the SA treatment, the seed meal expected to produce the most pronounced herbicidal effects due to the formation of SCN<sup>-</sup>. Ionic thiocyanate has a relatively short soil residence time, with 40-95% being lost within a six-day period in one study (Brown and Morra, 1993). Although 2-propenyl also has a short half-life of 20 to 60 hours (Borek et al., 1995), it is likely that the wet conditions for the second year resulted in retention of 2-propenyl ITC while the SCN<sup>-</sup>, being more water soluble and less volatile than 2-propenyl, was leached through the soil profile. Thus, it is likely that the SCN<sup>-</sup> was completely gone at the end of the 28-day-period after meal application and before planting and no longer a threat to weed emergence.

Despite the difference in environmental conditions, 3% BJ inhibited early season (first harvest) weed biomass relative to the no meal treatment in both years (Figures 3 and 5). The 3% BJ treatment had at least 75% lower average weed biomass (all species combined) for the first harvest in both years relative to the no meal treatment. Three percent BJ plots also had 74% lower weed biomass for the first harvest of pigweed during year two relative to the no meal amendment (Figure 5).

The lack of significant difference, however, between 3% BJ (high glucosinolate) and 3% BN (low glucosinolate), except for the second harvest of pigweed where 3% BN reduced weed biomass relative to 3% BJ, indicates that the glucosinolates may not be the only allelochemicals resulting in the reduction of weed biomass. Inhibition of lettuce seed germination by non-glucosinolate degradation products from BN seed meal occurred in a study by Brown and Morra

(1996). The authors concluded that a specific non-glucosinolate compound was probably responsible for inhibition of seed germination although one was not identified (Brown and Morra, 1996). Thus, it is possible that other allelopathic compounds in BN are responsible for the inhibition of weed biomass seen with the low glucosinolate meal.

#### Lettuce and beet emergence

Meal amendment did not inhibit seedling emergence in year one compared to that measured in the no-meal plots. In year two, all meals reduced emergence relative to the no-meal treatment and 3% BJ inhibited crop emergence by at least 58% relative to the other meals (Figure 8). Reduced stand counts in the second year in BJ-amended plots indicate that the 3% BJ meal application resulted in inhibition of plant emergence 28 days after meal amendment. This could be a result of 2-propenyl still being active during seed germination, thus inhibiting plant emergence. The difference seen between years with crop emergence may be a result of early season climatic conditions since irrigation later in the season would reduce variability in soil environmental conditions. The dry conditions of the first year likely increased volatilization of 2-propenyl ITC. It is also possible that the wet conditions of the second year increased hydrolysis of glucosinolates, creating an early flush of ITCs. The wet conditions may have further resulted in increased retention times of ITC (Borek et al., 1995) thus inhibiting crop emergence.

Thus, while our field study results do indicate that Brassicaceae seed meal can suppress weed biomass, this result was only evidenced with early season weeds. Because of the inability to control weeds later in the season and the natural high variability in environmental conditions and soil weed seed bank characteristics, the seed meal should not be used as a sole source of weed control. Our field study results also indicate inhibition of crop emergence by high glucosinolate seed meals suggesting that crop specific planting dates that reduce phytotoxicity must be established. The influence of environmental conditions on volatile ITC retention must also be better understood.

#### Laboratory Study

Not surprisingly, significantly greater amounts of N were mineralized in meal-mended soils than in the no meal treatment (Table 4) during the 28-d monitoring period as a result of the

low C:N ratios and high percentages of readily mineralizable protein in the meal (Wetter, 1965). The amount of N mineralized from Brassicaceae meal is superior to that reported from pig manure (Eneji et al., 2002). With an average C:N ratio of 10.8:1 for pig manure (compared to a range of 8.1 to 9.1:1 for seed meals used in this study) only 68% of the total available N in pig manure was mineralized after 8 weeks, whereas between 52 and 112% of the available N was mineralized within 21 d in our seed meal experiment. These results suggest that certain Brassicaceae seed meals offer organic growers a substantial amount of N for the initially critical period of vegetative growth.

Ammonium production in all BJ-and SA-amended soils significantly lagged behind that in both BN treatments (Figure 9). We propose that allelochemicals produced in BJ and SA meals caused inhibition of microbial activity in these experiments delaying N mineralization. 2-Propenyl ITC produced from glucosinolates in BJ meal is known to inhibit microbial activity (Vaughn 1999; Brown and Morra, 1997). Although the primary glucosinolate degradation product released by *S. alba* meal is not as biologically active as 2-propenyl ITC (Brown and Morra, 1993), adverse impacts of  $\text{SCN}^-$  on bacterial populations have been reported (Smith et al, 1946; Beekhuis, 1975).

By day 7 the statistical differences were between meal application rates rather than meal variety, indicating that glucosinolate hydrolysis products were no longer inhibiting the amount of N mineralized. Our data support the laboratory findings that 2-propenyl ITC as produced in BJ meal, has a relatively short half life ranging from 20 to 60 h depending on the soil (Borek et al., 1995). Concentrations of  $\text{SCN}^-$  are also expected to decrease relatively rapidly in soils (half life of 60 to 120 h), as a result of microbial degradation (Brown and Morra, 1993). Allelochemical influences on N mineralization were of a relatively short duration, thus explaining why inhibition was not observed in the field study.

The lower concentrations of  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N in BJ- and SA-amended treatments as compared to the BN treatments indicate that nitrification was inhibited by these two meals. Nitrifiers are some of the most sensitive of the physiological groups of bacteria to exotic chemicals (Schmidt, 1982) and as a result, may be particularly sensitive to glucosinolate-derived allelochemicals. This may help explain the fact that Kirkegaard et al. (1999) found a decrease in the number of  $\text{NH}_4^+$ -oxidizing bacteria present after a canola crop. 2-Propenyl ITC produced by BJ meal has been suggested to interfere with nitrifier activity (Bending and Lincoln, 2000),

possibly by directly affecting the size of the bacteria community responsible for nitrification. Also of interest is the early work of Smith et al. (1946) that demonstrated reductions of bacteria actively involved in the nitrification process with addition of  $\text{NH}_4\text{SCN}$  to soil. The sensitivity of nitrifiers to chemicals combined with the high level of toxic ITCs or  $\text{SCN}^-$  in some Brassicaceae seed meals makes it highly likely that inhibition of nitrification may occur with meal application. The inhibition of nitrification as noted here however, appears only transitory since no such effect was observed in field results.

The apparent decrease in N mineralization on day 28 for all meal treatments except 3% SA was largely a result of the decrease in  $\text{NH}_4^+$ , the dominant species present after day 21 (Figure 9a). Decreases in  $\text{NH}_4^+$ -N are caused most likely by increased nitrification followed by bacterial utilization of the resulting  $\text{NO}_3^-$ -N. Thus the amount of extractable  $\text{NO}_3^-$ -N reflects the amount of  $\text{NH}_4^+$  nitrified minus  $\text{NO}_3^-$  immobilized by the microbial community.

This relationship adequately explains all treatments except for 3% SA in which  $\text{NH}_4^+$ -N concentrations continue to increase. We hypothesize that  $\text{SCN}^-$  produced by SA meal inhibits nitrification for a longer time period than ITC produced by BJ meal. As a result, nitrification inhibition in the 3% SA treatment continues at least until day 28 and  $\text{NH}_4^+$ -N concentrations correspondingly continue to increase. Lesser amounts of  $\text{NO}_3^-$ -N are made available to the microbial community, limiting microbial population increases such that less immobilization occurs. With less immobilization, net amounts of nitrified products measured in soil extracts are within the same range as those determined for other meal-amended soils.

The latter observations illustrate a key point in extrapolating results from laboratory experiments to make predictions concerning potential field results. Laboratory incubation vessels do not allow for allelochemical dissipation within the soil environment. In the case of  $\text{SCN}^-$  in laboratory assays, this anion would remain in the zone in which mineralization and nitrification are actively occurring and hence, we observed nitrification inhibition. As we noted in the field, major impacts on N cycling by  $\text{SCN}^-$  produced in SA treatments were not obvious, most likely because this potential allelochemical was leached deeper into the soil profile where it had no impact. Similarly, ITC produced in BJ treatments appears to have little effect on N mineralization when monitored in laboratory assays past 21 days. However in field experiments, mineralization of not only N within the meal occurred, but allelochemicals released from the

meal in some as yet undetermined fashion stimulated mineralization of native soil organic matter.

#### Growth chamber

The results of the growth chamber experiment clearly indicate that SA can inhibit lettuce emergence if planted before 5 weeks after meal application (Figure 10). This corresponds with other literature suggesting that SA can inhibit plant emergence (Johansson, 1992). Our results suggest that SA can not only suppress, but also effectively inhibit lettuce emergence. This was evidenced by the lack of plant emergence between the 7 and 14 day counts (Figure 10). The lack of significant differences in lettuce emergence among treatments after week five indicates that the optimal planting date for lettuce is at least five weeks after meal amendment in laboratory studies (Figure 10). Since lettuce grows best under cooler temperatures, meal must be applied early enough in the season to avoid temperatures above optimum range. Early season application dates pose a problem in areas where the soil is too wet to work. Due to the known sensitivity of lettuce to ITC (Brown and Morra, 1996), this planting date should also be suitable for crops with larger seed size. It is important to note that the soils were kept moist through this study. Pronounced wet and dry periods experienced in the field may alter the planting date to some extent, due to the requirement of moisture for ITC production and its impact on ITC volatilization and degradation reactions. The results of the laboratory study confirm the field results from year two that indicate significant inhibition of crop emergence in SA and BN amended soils. The fact that no inhibition was noticed in the field study results from year one suggest that differences in climatic conditions can alter the activity of these allelochemicals in soils and that it may not be possible to apply the results of laboratory experiments to field situations.

Brassicaceae seed meal soil amendments may improve sustainability in organic agriculture by increasing plant available N. The seed meals have lower C:N ratios than green plant tissues, making them more useful as topically applied soil amendments in areas where cover crop tissues do not have sufficient time for decomposition. Our results indicate that certain Brassicaceae meals have the potential to increase crop yields, releasing plant-available N during critical periods of plant growth.

The field study results suggest that Brassicaceae meal, can be used as a method to control early season weeds. These results were most consistently seen with the 3% BJ amendment. The lack of significant differences in weed biomass between the low glucosinolate meal (BN) and high glucosinolate meal (BJ) may be due to the ability of 4-OH-indolyl-3-methyl found in BN meal to produce  $SCN^-$ . It may also suggest that factors other than glucosinolate content contribute to the reduction of weed biomass following meal application. Single applications of meal do not appear to adequately control weeds throughout the growing season and may result in higher late-season weed biomass due to increases in plant available nitrogen. For these reasons, meal may not be a suitable method of weed control in all systems.

Due to the dependence of ITC formation and degradation on soil moisture conditions, climatic conditions and irrigation practices must also be accounted for when determining planting dates. Further understanding of the mode of action and persistence of glucosinolate secondary products under field conditions needs to be gained to better predict planting dates that avoid crop inhibition.

### Outreach

Data has been posted on the following website: <http://soils.ag.uidaho.edu/swm/Organic.htm>

### Presentations:

Johnson-Maynard, J.L. Carbon research at the University of Idaho. Idaho Carbon Sequestration Advisory Committee meeting. Moscow, Idaho. July 10, 2002.

Arnold, A., J.L. Johnson-Maynard, and M. Morra. 2003. Application of Brassica Meal for Disease Control and Improved Nitrogen Fertility in Organic Farming Systems. Soil Science Society of America Annual Meetings, Denver, Colorado. Nov. 2-6.

### Abstracts:

Arnold, A., J.L. Johnson-Maynard, and M. Morra. 2003. Application of Brassica Meal for Disease Control and Improved Nitrogen Fertility in Organic Farming Systems. ASA-Soil Science Society of America. Denver, Colorado. November 2-6 (available on CD-rom).

#### Articles:

Williams, C. 2002. News flash- Organic research at UI. Rural Roots: The Inland Northwest Community Food Systems Association. Vol. 4, number 3-4, page 16.

Johnson-Maynard, J. 2002. Can *Brassica* species improve the sustainability of organic farming systems? Rural Roots: The Inland Northwest Community Food Systems Association. Vol. 4 number 3-4, page 16.

Rice, A. J.L. Johnson-Maynard, M.Morra and L. Dandurand. Brassicaceae seed meal as a soil amendment to improve plant-available nitrogen and yields in organic farming systems. In preparation.

Rice, A. J.L. Johnson-Maynard, D.C. Thill, and M. Morra. Allelopathic potential and influence of Brassicaceae seed meal on weed growth and plant emergence In preparation.

The collaborative efforts initiated by this grant led to additional interaction with Mary Jane Butters of Paradise Farm Organics that resulted in submission of an Idaho Specialty Crop Grant netting \$50,000. The funding is to be used to purchase a seed press and begin producing meal products on-site at Paradise Farm Organics. This facility will be used for educational and outreach purposes and will serve to promote our work. It will be highly visible to organic growers as part of field tours and other on-site activities at Paradise Farm Organics.

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