Seed Meals from Brassicaceae Oilseed Crops as Soil Amendments: Influence on Carrot Growth, Microbial Biomass Nitrogen, and Nitrogen Mineralization

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Abstract. Brassicaceae seed meals (BSMs) average 6% nitrogen (N) by weight and contain glucosinolates (GLSs) that produce biologically active compounds. A two-season field study was initiated to determine how *Brassica juncea* L., *Brassica napus* L., and *Sinapis alba* L. seed meals, each with different glucosinolate profiles, alter carrot (*Daucus carota* L. subsp. *sativus*) growth, microbial biomass N (MBN), and soil N mineralization. BSM applications of 1 and 2 t·ha⁻¹ 36 days before planting did not influence carrot emergence, whereas carrot emergence decreased up to 40% in *S. alba* treatments seeded 15 days after BSM application. Crop quality was unaffected by BSM treatments and total fresh market yields were equal to or higher than the unamended controls in both years. At 4 and 8 days after seed meal application, MBN in the high-GLS *B. juncea* and *S. alba* treatments was 48% to 67% lower than in the low-GLS *B. napus* treatment. Seasonal apparent net N mineralized expressed as a percentage of the total N applied in the seed meals was unaffected by glucosinolate concentration and ranged from 30% to 81% across both years. BSMs can be used to increase soil inorganic N and carrot yields, but crop phytotoxicity is possible depending on the meal and its respective glucosinolate content. GLS degradation products inhibit microbial N uptake in the short term, but longer-term N availability is not compromised.

Brassicaceae seed meals (BSMs) are byproducts of edible and industrial-grade oil production from crops such as canola/rapeseed (*Brassica napus* L.) and mustard (e.g., *Brassica juncea* L. and *Sinapis alba* L.). Brassicaceae oilseeds contain 30% to 40% oil by weight; hence, an increase in oilseed production will result in seed meal availability. BSMs currently have limited market value, except for canola meal as an animal feed, and establishing appropriate and profitable applications is important to sustaining edible-oil, biofuel, and biolubricant production from Brassicaceae oilseed crops (Bender, 1999).

One prospective application of BSMs is as soil amendments to control agricultural pests.

Plants in the Brassicaceae contain compounds called glucosinolates (GLSs), which on enzymatic hydrolysis release biologically active products (Brown and Morra, 1997; Chew, 1988; Fenwick et al., 1983; Matthiesen and Kirkegaard, 2006; Rosa et al., 1997; Vaughn et al., 2006). Glucosinolates, most concentrated in the seed, are preserved in the seed meal until the addition of water initiates glucosinolate hydrolysis and the production of biocidal products (Morra, 2004). Researchers have documented the efficacy of BSMs as biopesticides to control weeds (Ascard and Jonasson, 1991; Rice et al., 2007; Vaughn et al., 2006), insect pests (Elberson et al., 1996, 1997), nematodes (Walker, 1996), and pathogens (Chung et al., 2002; Mazzola et al., 2001; Smolinska et al., 1997), but no studies have compared BSMs from different species as sources of nitrogen (N) for vegetable crop production.

Brassicaceae seed meals contain 5% to 6% N by weight and have C:N ratios of ≈8:1 (Gale et al., 2006), thus potentially serving as an organic source of N in agricultural production systems. The N contents and C:N ratios of BSMs are similar to those of cottonseed and soybean seed meals proven to be effective at increasing N availability in organic agricultural systems (Hall and Sullivan, 2001). Rapeseed seed meal applied as a fertilizer to wheat, barley, and sugar beets increased N uptake and improved yields and

crop quality (Kucke, 1993). Researchers have observed elevated N content in apple leaves and increased total soil N in greenhouse and orchard settings when using *B. napus* seed meals as soil amendments (Mazzola et al., 2001).

Because BSMs have comparable N contents and C:N ratios, one would expect them to have similar effects on N mineralization and soil N cycling. However, GLS degradation products exhibit a degree of biocidal activity controlled by GLS type and concentration (Brown and Morra, 1997; Vaughn, 1999). BSMs with different GLS profiles may therefore not only have mixed effects on pest or crop species, but may also differentially affect the microbially mediated soil N cycle. Our objectives were to assess the effects of three Brassicaceae seed meals with different GLS profiles and similar N contents on plant growth and seasonal N mineralization in carrot production. We are specifically targeting the potential use of Brassicaceae seed meals in organic agricultural systems. Carrot (*Daucus carota* L. subsp. *sativus*) was chosen as the test crop because of its potentially greater tolerance to glucosinolate hydrolysis products than other high-value vegetable crops in which Brassicaceae meals are likely to be used (Stiehl and Bible, 1989). Evaluation parameters included carrot emergence, yield, quality, and N content as well as microbial biomass N and soil inorganic N.

Materials and Methods

Study site and experimental design. A two-season field study was initiated at the University of Idaho Plant Science Farm in Moscow, ID (lat. 46°43'25" N, long. 116°57'30" W) within a section of the farm restricted to organic agriculture. The soil at the experimental site was a Latahco silt loam (McDole and Peterson, 1989) and contained 20% sand, 60% silt, 20% clay, and 2.7% organic matter. The soil pH (1:1, soil:water) was 6.3 and the cation exchange capacity was 18.7 cmol_c·kg⁻¹ soil in the 0- to 20-cm depth. Concentrations of soil preplant macro- and micronutrients were similar in both years and adequate to support carrot growth: phosphorus 56 (weak Bray); potassium 217, calcium 1570, magnesium 328, sulfur 11 (ammonium acetate, pH 7.0); manganese 14, iron 46, copper 1.8 (DTPA); boron 0.4, and zinc 1.8 (sorbitol DTPA) mg·kg⁻¹ soil (Fritz et al., 2006; Ontario Ministry of Agriculture, Food and Rural Affairs, 2006).

Plots were moved after the first year to avoid any residual effects of meal application. Spring canola (*B. napus*) was grown before 2005 and hard red spring wheat (*Triticum aestivum*) before the 2006 study. Residual effects of canola glucosinolates on 2005 treatments were considered inconsequential compared with the amount of glucosinolate added in the seed meals, because canola contains low glucosinolate concentrations, glucosinolates are rapidly hydrolyzed or degraded, and seed was harvested from the plots. The experimental design was a

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randomized complete block with seven treatments and four replications. Treatments included three BSMs applied at 1 and 2 t·ha⁻¹ rates and an unamended control. The BSMs used as soil amendments were obtained from three locally adapted oilseed varieties: *B. napus* 'Sunrise' (Brown et al., 1998a), *B. juncea* 'Pacific Gold' (Brown et al., 2004), and *S. alba* 'IdaGold' (Brown et al., 1998b).

Seed meal characterization and application. Seed of each variety was cold pressed removing ≈90% of the oil (Peterson et al., 1983). For glucosinolate analysis only, subsamples of the cold-pressed BSMs were extracted with petroleum ether to remove any remaining oil. Methanol extracts of these defatted samples were analyzed for GLS concentrations using a Waters high-performance liquid chromatography separation module coupled with a Waters 996 photodiode array detector and a Thermabeam Mass Detector according to previously reported methods (Borek and Morra, 2005).

Total carbon, N, and sulfur concentrations in the cold-pressed BSMs were determined through dry combustion of a finely ground sample in an Elementar varioMAX CNS analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). Total concentrations of other essential elements were determined in nitric acid digests of the BSMs using an IRIS inductively coupled plasma spectrometer (Thermo Electron Corp., Madison, WI).

On 31 May 2005 and 17 May 2006, a tractor-mounted rototiller was used to till to a depth of 15 cm and form four 1-m wide beds (replications) separated by 0.6-m wide paths. Within each bed, seven 1 × 2-m plots were established. *B. napus*, *B. juncea*, and *S. alba* seed meals were uniformly applied by hand to the soil surface on 8 June 2005 and 18 May 2006 at 1 and 2 t·ha⁻¹ and incorporated to a depth of 2 cm with a landscaping rake. An unamended control plot was retained in each replicate. Immediately after meal incorporation in both years, 27 mm of drip irrigation water was applied to the plots and a tractor-mounted sprayer was used to deliver an additional 3 mm of water to assure complete wetting of the seed meal. In 2005, during the 36-d period from seed meal application to seeding, 36 mm of precipitation was recorded and plots were irrigated with a total of 419 mm of water to accommodate auxiliary research projects (Hansson et al., 2008). In 2006, during the 15-d period between seed meal application and seeding, 62 mm of precipitation was recorded and no additional irrigation was applied.

Agronomics. Pelleted seed of the 'Nelson' carrot variety (*D. carota* L. var. *sativus*) was used (Johnny's Selected Seeds, Winslow, ME). The 'Nelson' variety is a popular Nantes-type carrot variety valued for its short period to maturity and its performance in high-temperature summer conditions. Carrots were seeded at a rate of 78 seeds/m using an EarthWay precision seeder fitted with a custom seeding plate (EarthWay Products, Bristol, IN). Four rows of carrots were seeded in each replicate bed with a between-row

spacing of 25 cm. Each carrot row was located 12 cm from the drip irrigation tubing. To minimize soil crusting and associated emergence inhibition, a 1-cm thick by 3-cm wide layer of a peatmoss, perlite, gypsum, and limestone mixture (Sun Gro Horticulture, Bellevue, WA) was uniformly placed over the seeded carrot rows. In 2005, carrots were seeded on 14 July, 36 d after meal application. Based on the lack of emergence inhibition observed in 2005, carrots were seeded on 1 June 2006, 15 d after BSM application. All plots were hand-weeded immediately before seeding and as necessary throughout the growing season.

In both years, carrot emergence was measured 21 d after seeding by counting the number of carrots in 2 m of the two inside rows within each plot. Results are expressed on a meter-row basis. After emergence counts, carrots were thinned to a 5-cm spacing to minimize plant competition and facilitate proper root development.

A pressure-compensated drip irrigation system was constructed to ensure quantitative and reliable irrigation. Two parallel lengths of emitter tubing were installed in each replicate bed with each length of tubing ≈25 cm from the center of the bed (Hansson et al., 2008). Emitters located every 23 cm along the tubing length delivered an average of 1.9 L·h⁻¹ per emitter. A 1-h irrigation period resulted in an apparent irrigation rate of 18 mm·h⁻¹ across the plot. In both years, soil moisture within the 0- to 20-cm depth was measured every 4 to 6 d (≈24 h after each irrigation cycle) using gravimetric and tensiometric methods. A soil water characteristic curve was developed in the laboratory using field soil to relate gravimetric and tensiometric data.

After carrots were planted in both years, irrigation was managed to retain a moisture content of at least 50% of the available water capacity (AWC) for the 0- to 20-cm depth and provide soil moisture conditions optimum for carrot growth. The AWC was determined to be 0.20 g H₂O/g soil, and a minimum allowable moisture depletion threshold of 0.10 g H₂O/g soil was established. In 2005, plots received 1139 mm of water through irrigation and precipitation for the period from BSM application to carrot harvest with a minimum soil moisture content of 0.15 g H₂O/g soil and a mean of 0.22 g H₂O/g soil. In 2006, the plots received 743 mm of water through irrigation and precipitation for the period from BSM application to harvest with a minimum soil moisture content of 0.12 g H₂O/g soil and a mean of 0.22 g H₂O/g soil. Meteorological data were collected at a semiautomated weather monitoring station located 500 m from the study site.

Microbial biomass nitrogen. In 2006, microbial biomass nitrogen (MBN) was measured in the 2 t·ha⁻¹ BSM treatments and the control to assess the impact of different BSMs on the microbial N pool. Microbial biomass N was measured on six dates: 18 May (pre-BSM), 22 May, 26 May, 1 June, 14

July, and 15 Aug. Three samples from the 0- to 5-cm depth (each ≈284 cm³) were collected from each plot and composited. Subsamples were dried for 24 h at 110 °C to determine soil moisture content and remaining samples were stored at 4 °C until analysis. Within 3 d of collection, soils were processed for microbial biomass N by the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). After the extraction procedure, all samples were stored at -20 °C. Thawed extracts were filtered through 0.2-μm syringe filters into glass vials and capped with septum-fitted lids. Samples were stored at 4 °C for less than 24 h until analysis for total K₂ SO₄-extractable N by combustion on a Shimadzu TN analyzer (Shimadzu Scientific Instr., Kyoto, Japan). MBN was calculated using a *k*_{EN} value of 2.22 (Brookes et al., 1985).

Soil and plant nitrogen. Soil samples were collected from the 0- to 5-, 5- to 10-, 10- to 20-, 20- to 30-, and 30- to 50-cm depths before BSM application (3 June 2005; 18 May 2006) and at harvest (4 Oct. 2005; 22 Aug. 2006). Four core samples from each depth increment were combined to provide a single composite sample for each depth in each plot. Soils were air-dried at ambient laboratory temperature until a constant weight was established, ground with a mortar and pestle, and sieved to obtain the less than 2-mm size fraction. Soil extracts for inorganic N analyses were obtained by adding 10 g soil and 80 mL 2 M KCl to polyethylene bottles followed by reciprocal shaking for 1 h. Extracts were filtered through Whatman 45 filters and stored at -20 °C until analysis. Extractable ammonium (NH₄⁺)- and nitrate (NO₃⁻)-N concentrations were determined colorimetrically using a Lachat rapid flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). Inorganic N is presented as 0- to 50-cm depth-weighted average concentrations to represent total inorganic N in the root zone.

Carrots were harvested by hand at a size typical for fresh-market sale (≈15-cm length) on 6 Oct. 2005 and 22 Aug. 2006. In 2005, all carrots within a 0.5-m length of the two inner carrot rows were harvested; thus, a total 1 m-row of sample was collected. In 2006, the sample size was expanded to a 1-m length of the two inside rows for a total 2-m row of sample. Carrots were graded as marketable or nonmarketable based on size and visually detectable deformities. Nonmarketable carrots were defined as carrots with roots less than 2 cm in diameter measured 2 cm from the top of the root crown or roots with visually detectable deformities such as forking, hairiness, or pest damage. Fresh weights of shoots and washed roots were obtained in the field. Subsamples of roots and shoots from six marketable carrots from each plot were dried at 60 °C until a constant weight was reached to determine the root and shoot moisture and N contents. Dried tissue samples were ground to pass a 1.0-mm (20 mesh) screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) and 300-mg

samples were analyzed for total N by dry combustion with an Elementar varioMAX CNS analyzer (Elementar Americas, Inc.).

Apparent net nitrogen mineralized (ANM) (grams N/m²) from before BSM application to harvest was calculated using the following equation:

$$\text{ANM} = \Delta N_{\text{inorg_soil}} + N_{\text{plant}} \quad (1)$$

where $\Delta N_{\text{inorg_soil}}$ (grams N/m²) is the change in soil inorganic N (NO₃⁻-N and NH₄⁺-N) concentrations from before BSM application to harvest and N_{plant} (grams N/m²) is the total N in the carrot root and shoot biomass. Soil inorganic N concentrations used in calculating ANM are based on 0- to 50-cm depth-weighted averages converted to an areal basis using the measured average soil bulk density of 1.30 g·cm⁻³.

The percent of total seed meal N mineralized in the treatments (%ANM) was calculated using the following equation:

$$\% \text{ANM} = \frac{(\text{ANM}_{\text{BSM}} - \text{ANM}_{\text{control}})}{N_{\text{BSM}}} \times 100 \quad (2)$$

where ANM_{BSM} is the ANM in a BSM treatment, $\text{ANM}_{\text{control}}$ is the ANM in the unamended control, and N_{BSM} is total seed meal N applied to the soil in BSM treatments. We assumed ANM in the control plots was background mineralization in the BSM treatments.

Statistics. Data were analyzed using SAS 9.1 and a general linear statistical model. Analysis of variance was used to detect significant treatment effects ($P \leq 0.05$) and means were separated by a protected Fischer's least significant difference test ($P < 0.05$). Soil inorganic N, ANM, and MBN data were log-transformed to normalize the distributions, and untransformed means are presented in the "Results." Covariate analysis was also used to determine potential covariate effects.

Results

Seed meal characterization. The three varieties of BSMs differed in both the amount and type of GLSs present. The dominant GLS measured in *B. juncea* seed meal was 2-propenyl (152.0 $\mu\text{mol}\cdot\text{g}^{-1}$). *Sinapis alba* seed meal contained mainly 4-hydroxybenzyl (156.8 $\mu\text{mol}\cdot\text{g}^{-1}$). A greater variety of GLSs lower in concentration were measured in *B. napus* seed meal, including 4-hydroxy-3-indolylmethyl (10.0 $\mu\text{mol}\cdot\text{g}^{-1}$), 3-butenyl (2.9 $\mu\text{mol}\cdot\text{g}^{-1}$), 2-hydroxy-3-butenyl (1.3 $\mu\text{mol}\cdot\text{g}^{-1}$), and 4-methoxy-3-indolylmethyl (0.5 $\mu\text{mol}\cdot\text{g}^{-1}$).

Brassicaceae seed meals averaged 50% carbon, 5.9% N, and 1.3% phosphorus by weight (Table 1). *B. napus* had less than half the sulfur as compared with the two mustards. The concentration of micronutrients iron, zinc, sodium, boron, and copper were very similar among BSMs (Table 1). The C:N ratios of the BSMs (*B. juncea*, 8.2:1; *B. napus*, 8.7:1; *S. alba*, 8.2:1) were similar and averaged 8.4:1.

Carrot emergence, nitrogen content, and yield. In 2005, seedling emergence and total number of carrots harvested did not differ among treatments (Table 2). No differences were detected in shoot yields (16% of total weight reported); therefore, root and shoot yields were combined to provide a simplified presentation of yield results and allow comparison with published average fresh market yields of bunched carrots. Total root and shoot yields (nonmarketable and marketable combined) and total marketable yields (roots and shoots combined) were not different across treatments (Table 2). There were no differences in carrots graded as nonmarketable on a number or weight basis (data not shown).

In the 2006 season, all BSM treatments resulted in lower carrot emergence than in the control except *B. juncea* 1 t·ha⁻¹ (Table 2). The most substantial emergence inhibition was in the *S. alba* treatments, in which emergence counts in the 1 t·ha⁻¹ and 2 t·ha⁻¹ treatments were equivalent and $\approx 60\%$ of those measured in the control. Only the 2 t·ha⁻¹ *B. napus* treatment produced emergence inhibition similar to that recorded for the two seed meal rates of *S. alba*. As a result of manual stand thinning after emergence counts, the total number of carrots harvested was not different among treatments (Table 2).

At harvest in 2006, no differences in shoot yields (17% of total weight reported) were detected; thus, root and shoot yields were combined. Total root and shoot yields (marketable and nonmarketable combined) in all BSM treatments were higher than in the control except for the *S. alba* 1 t·ha⁻¹ treatment (Table 2). Marketable yields (roots and shoots combined) were not different among treatments (Table 2). There were no differences in carrots graded as nonmarketable on a number or weight basis (data not shown).

In 2005, percentages of N in the root tissues and both root and shoot N uptake did not differ among all treatments and the control (Table 3). The only measured plant N parameter that differed among treatments was shoot N percentage. Percent N in shoots of both *S. alba* treatments and in the *B. napus* 2 t·ha⁻¹ treatment were higher than in carrot shoots harvested from the control plots (Table 3).

Percentages of N in the roots and shoots and root N uptake were not different across all treatments (Table 3) in 2006. Differences were detected in shoot N uptake with all BSM treatments resulting in higher shoot N uptake compared with that measured in the control except for the *S. alba* 1 t·ha⁻¹ treatment (Table 3).

Microbial biomass nitrogen. Background MBN concentrations measured on Day 0 (18 May 2006) immediately before BSM application were similar across the study site (Fig. 1). Microbial biomass N concentrations in all BSM treatments were greater than in the control on Days 4, 8, and 14. Differences in MBN among BSM treatments were detected on Days 4 and 8 (Fig. 1). On Day 4, MBN in the *B. napus* treatment (318.0 $\mu\text{g N/g soil}$)

was greater than MBN measured in *B. juncea* (164.1 $\mu\text{g N/g soil}$) and *S. alba* (104.2 $\mu\text{g N/g soil}$) treatments, which were similar. On Day 8, MBN in the *B. napus* (211.6 $\mu\text{g N/g soil}$) and *B. juncea* (191.8 $\mu\text{g N/g soil}$) treatments were not different but were higher than in the *S. alba* treatment (110.5 $\mu\text{g N/g soil}$). By Day 14, MBN measured in all BSM treatments were similar (195.5 to 257.6 $\mu\text{g N/g soil}$), but all remained greater than in the control (47.7 $\mu\text{g N/g soil}$). MBN in all treatments, including the control, were similar by Day 43 (Fig. 1). Microbial biomass N measured at any given day did not have a covariate effect on seasonal ANM ($P = 0.25$).

Soil inorganic nitrogen and apparent net nitrogen mineralized. On 3 June 2005 before BSM application, the 0- to 50-cm depth-weighted average NH₄⁺-N concentrations across the study site ranged from 2.3 to 3.5 mg NH₄⁺-N/kg soil and NO₃⁻-N concentrations ranged from 6.1 to 11.1 mg·kg⁻¹ NO₃⁻-N soil (Fig. 2A). Although the total inorganic N concentrations for the 0- to 50-cm depth were different across untreated plots (Fig. 2A), initial inorganic N concentrations did not have a covariate effect on seasonal ANM or plant parameters ($P = 0.87$).

At harvest on 4 Oct. 2005, total soil inorganic N concentrations for the 0- to 50-cm depth ranged from 11.4 to 18.9 mg inorganic N/kg soil in BSM treatments, whereas the control had 6.4 mg inorganic N/kg soil. Nitrate in the BSM treatments ranged from 8.3 to 15.9 mg NO₃⁻-N/kg soil compared with 4.5 mg NO₃⁻-N/kg soil in the control. Both total inorganic N and NO₃⁻-N concentrations in all BSM treatments were higher than in the control (Fig. 2B). Among BSM treatments, differences were limited to lower NO₃⁻-N and total inorganic N concentrations in the *S. alba* 2 t·ha⁻¹ treatment compared with the *B. napus* 2 t·ha⁻¹ treatment. Ammonium concentrations were not different across treatments (1.8 to 3.2 mg NH₄⁺-N/kg soil) (Fig. 2B).

In 2006, total soil inorganic N concentrations on 18 May, before BSM application, were uniform by depth across the study site with no differences in NH₄⁺-N or NO₃⁻-N (Fig. 3A). The 0- to 50-cm weighted average NH₄⁺-N concentrations ranged from 6.6 to 8.0 mg NH₄⁺-N/kg soil and NO₃⁻-N concentrations ranged from 2.8 to 3.3 mg NO₃⁻-N/kg soil (Fig. 3A).

At harvest on 22 Aug. 2006, average inorganic N concentrations in the 0- to 50-cm depth in all BSM 2 t·ha⁻¹ (12.6 to 17.7 mg inorganic N/kg soil) and in the *B. napus* 1 t·ha⁻¹ treatments were higher than in the control (6.1 mg inorganic N/kg soil) (Fig. 3B). Within the same rate, inorganic N concentrations were not different among BSM treatments. Nitrate concentrations in all BSM treatments (3.2 to 10.9 mg NO₃⁻-N/kg soil) were higher than in the control (1.8 mg NO₃⁻-N/kg soil) (Fig. 3B). Ammonium concentrations were not different across all treatments (4.3 to 6.8 mg NH₄⁺-N/kg soil) (Fig. 3B).

Table 1. Elemental concentrations in *B. juncea*, *B. napus*, and *S. alba* seed meals used in 2005 and 2006 field experiments.

Seed meal	Carbon	Nitrogen	Phosphorus	Potassium	Sulfur	Calcium	Magnesium	Iron	Zinc	Sodium	Boron	Copper
	(% dry wt)											
<i>B. juncea</i>	49.3	6.0	1.3	1.0	1.8	0.43	0.50	0.012	0.006	0.003	0.002	0.001
<i>B. napus</i>	50.3	5.8	1.4	1.5	0.7	0.42	0.44	0.010	0.006	0.002	0.002	0.001
<i>S. alba</i>	49.0	6.0	1.1	1.0	1.8	0.48	0.38	0.012	0.007	0.002	0.001	0.001

Table 2. Carrot emergence, total harvest count, total yield, and total marketable yield for 2005 and 2006.^z

Treatment	Seedling emergence (no. m-row ⁻¹)	Total harvest count ^y (no. m ⁻²)	Total yield ^x (g·m ⁻²)	Total marketable yield ^w (g·m ⁻²)
2005				
<i>B. juncea</i> 2 t·ha ⁻¹	35.0	66	4,542	4,307
<i>B. napus</i> 2 t·ha ⁻¹	35.0	65	4,289	4,144
<i>S. alba</i> 2 t·ha ⁻¹	33.0	61	3,952	4,005
<i>B. juncea</i> 1 t·ha ⁻¹	30.3	54	3,447	3,388
<i>B. napus</i> 1 t·ha ⁻¹	33.3	62	3,830	3,687
<i>S. alba</i> 1 t·ha ⁻¹	31.5	61	3,785	3,698
Control	36.5	78	4,482	4,431
LSD _{0.05}	NS	NS	NS	NS
2006				
<i>B. juncea</i> 2 t·ha ⁻¹	35.6 c	59	4,046 a	3,617
<i>B. napus</i> 2 t·ha ⁻¹	32.2 cd	59	4,055 a	3,220
<i>S. alba</i> 2 t·ha ⁻¹	26.3 d	48	3,719 a	2,825
<i>B. juncea</i> 1 t·ha ⁻¹	41.8 ab	58	3,648 a	3,314
<i>B. napus</i> 1 t·ha ⁻¹	38.0 bc	62	3,796 a	2,984
<i>S. alba</i> 1 t·ha ⁻¹	27.7 d	48	3,130 b	2,342
Control	44.8 a	58	2,881 b	2,374
LSD _{0.05}	6.15	NS	514.1	NS

^zWithin the same column of the same year, means with different letters are significantly different ($P \leq 0.05$). Means within the same column of the same year without letters are not significantly different (NS) ($P \leq 0.05$).

^yIncludes both marketable and nonmarketable carrots.

^xTotal fresh weight of marketable and nonmarketable roots and shoots.

^wTotal fresh weight of marketable roots and shoots.

LSD = least significant difference.

Table 3. Root and shoot percent nitrogen (N) and root and shoot N uptake for 2005 and 2006.^z

Treatment	Root N (%)	Shoot N (%)	Root N uptake ^y (grams N/m ²)	Shoot N uptake ^y (grams N/m ²)
2005				
<i>B. juncea</i> 2 t·ha ⁻¹	0.99	2.85 bcd	4.87	6.70
<i>B. napus</i> 2 t·ha ⁻¹	0.99	2.96 abc	4.53	7.15
<i>S. alba</i> 2 t·ha ⁻¹	1.09	3.02 ab	4.76	6.39
<i>B. juncea</i> 1 t·ha ⁻¹	1.08	2.91 bcd	4.04	5.34
<i>B. napus</i> 1 t·ha ⁻¹	0.98	2.84 cd	4.14	5.55
<i>S. alba</i> 1 t·ha ⁻¹	1.07	3.08 a	4.44	6.75
Control	0.97	2.74 d	4.74	6.31
LSD _{0.05}	NS	0.13	NS	NS
2006				
<i>B. juncea</i> 2 t·ha ⁻¹	0.67	2.20	2.71	4.32 a
<i>B. napus</i> 2 t·ha ⁻¹	0.76	2.18	3.12	4.41 a
<i>S. alba</i> 2 t·ha ⁻¹	0.66	2.10	2.44	3.88 ab
<i>B. juncea</i> 1 t·ha ⁻¹	0.68	2.16	2.47	3.92 ab
<i>B. napus</i> 1 t·ha ⁻¹	0.76	2.14	2.88	3.96 ab
<i>S. alba</i> 1 t·ha ⁻¹	0.76	2.33	2.39	3.48 bc
Control	0.68	2.17	1.94	3.18 c
LSD _{0.05}	NS	NS	NS	0.64

^zWithin the same column of the same year, means with different letters are significantly different ($P \leq 0.05$). Means within the same column of the same year without letters are not significantly different (NS) ($P \leq 0.05$).

^yIncludes marketable and nonmarketable roots and shoots.

LSD = least significant difference.

In 2005, ANM during the 123-d period from 3 June (pre-BSM application) to 4 Oct. (harvest) ranged from 8.3 g N/m² in the unamended control to 17.8 g N/m² in the *B. napus* 2 t·ha⁻¹ treatment (Table 4). Although there was a trend for higher ANM across BSM treatments (11.3 to 17.8 g N/m²), only the *B. napus* 2 t·ha⁻¹, *B. juncea* 2 t·ha⁻¹, and *S. alba* 1 t·ha⁻¹ treatments resulted in higher

ANM values than that measured in the control (8.3 g N/m²) (Table 4). Across both rates, ANM in all BSM treatments was similar except for the *B. napus* 2 t·ha⁻¹ treatment (Table 4). Total plant N uptake (root and shoot N uptake combined) did not differ across all treatments (Table 4). The net changes in soil inorganic N from before BSM application to harvest ($\Delta N_{\text{inorg-soil}}$) in all

BSM treatments (1.8 to 6.3 g N/m²) were higher than that measured in the control (−2.4 g N/m²) (Table 4). In 2005, ANM expressed as a percent of total seed meal N applied in each BSM treatment (%ANM) ranged from 30% to 71% (Fig. 4).

For the 96-d period from 18 May (pre-BSM application) to 22 Aug. 2006 (harvest), ANM ranged from 2.3 g N/m² in the control to 11.4 g N/m² in both the *S. alba* and *B. juncea* 2 t·ha⁻¹ treatments (Table 4). In all BSM treatments, ANM was higher than in the control. Plant N uptake in all BSM treatments was higher than in the control except in the *S. alba* 1 t·ha⁻¹ treatment (Table 4). The net changes in soil inorganic N from before BSM application to harvest ($\Delta N_{\text{inorg-soil}}$) in the BSM 2 t·ha⁻¹ treatments (2.8 to 6.6 g N/m²) were similar and all were higher than in the control (−2.6 g N/m²) (Table 4). The $\Delta N_{\text{inorg-soil}}$ in the BSM 1 t·ha⁻¹ treatments (−0.1 to 1.5 g N/m²) were similar and not different from the control (Table 4). The ANM expressed as a percentage of total seed meal N added ranged from 55% to 81% across all BSM 2006 treatments (Fig. 4).

Discussion

Seed meal characterization. In *B. juncea* seed meal, the dominant GLS releases 2-propenyl isothiocyanate (ITC) on enzymatic hydrolysis (Larsen, 1981), a compound with broad-spectrum toxicity to microorganisms, insects, and plants (Brown and Morra, 1997; Norsworthy and Meehan, 2005; Vaughn et al., 2006). The dominant GLS in *S. alba* BSM hydrolyzes to unstable 4 OH-benzyl ITC that subsequently produces ionic thiocyanate (SCN⁻) (Borek and Morra, 2005; Kawakishi and Muramatsu, 1966). *B. napus* seed meal was low in total GLSs compared with the other two BSMs, but it still contained 10.5 μmol·g⁻¹ of indolylic GLSs that produce SCN⁻ (Brown and Morra, 1993). SCN⁻ is toxic to microorganisms and plants (Ahlgren et al., 1951; Ju et al., 1983; Smith et al., 1945; Stiehl and Bible, 1989). The release of 2-propenyl ITC and SCN⁻ from their respective GLS precursors in soil has been documented (Borek et al., 1994; Brown et al., 1991; Hansson et al., 2008).

The similar elemental profiles of the BSMs (Table 1) facilitate comparison of how different GSL degradation products affect plant growth and N mineralization. The average C:N ratio of 8.4:1 is less than the accepted critical value of 20:1, below which net production of inorganic N will occur when organic amendments are added to soil (Coyne, 1999; Sylvia et al., 2005).

Emergence inhibition. Emergence inhibition of various crop and weed species has

Fig. 1. Microbial biomass nitrogen measured in 2 t-ha⁻¹ seed meal treatments and the control in 2006. Seed meals and irrigation were applied on Day 0 (18 May) immediately after soil samples were collected. On the same day, significant differences among means are indicated by different letters, and no significant differences among means within a day are indicated by ns ($P \leq 0.05$).

been observed when BSMs are amended to greenhouse and field soils (Boydston et al., 2008; Rice et al., 2007; Vaughn et al., 2006). We suggest that 2-propenyl ITC and SCN⁻ released from GLSs found in BSMs were the dominant causes of emergence inhibition in 2006 (Table 2). Greater emergence inhibition was observed in *S. alba* than in *B. juncea* treatments across both rates (Table 2), most likely because of different glucosinolate types in the meals. Differences in ITC and SCN⁻ release from the tissues and dispersal and persistence of these compounds in soil all contribute to differential effects on emergence. Release efficiencies from Brassicaceae seed meals are controversial and difficult to predict (Brown et al., 1991; Hansson et al., 2008). Dispersal of the two compounds undoubtedly differs given that SCN⁻ will move by way of diffusion and mass flow, whereas 2-propenyl ITC is a relatively volatile and hydrophobic compound (Borek et al., 1995). Surface application with only shallow incorporation of BSMs used in this experiment likely favored volatile losses of 2-propenyl ITC, whereas leaching losses of SCN⁻ were limited by only 62 mm of precipitation and irrigation in the 15-d period between BSM application and seeding. Persistence differences of the two compounds may also contribute to observed results because a longer half-life for SCN⁻ in soil compared with 2-propenyl ITC has been reported (Borek et al., 1995; Brown and Morra, 1993).

We also observed carrot emergence inhibition in soils treated with the low-GLS *B. napus* seed meal, even when applied at only 1 t-ha⁻¹ (Table 2). Carrot emergence in the *B. napus* 2 t-ha⁻¹ treatment was not different from that in the *S. alba* 2 t-ha⁻¹ treatment, although the amount of SCN⁻ expected from *B. napus* seed meal was less than 10% of the SCN⁻ anticipated from *S. alba* seed meal.

Fig. 2. Depth-weighted average (0- to 50-cm) total soil inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$), $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ concentrations on (A) 3 June 2005 before seed meal application and (B) 4 Oct. 2005 at harvest 123 d after *B. juncea* (BJ), *B. napus* (BN), or *S. alba* (SA) seed meal application at 1 (1) or 2 (2) t-ha⁻¹. Different letters indicate significant differences for total inorganic nitrogen within a sample date ($P \leq 0.05$).

Fig. 3. Depth-weighted average (0- to 50-cm) total soil inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$), $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ concentrations on (A) 18 May 2006 before seed meal application and (B) 22 Aug. 2006 at harvest 96 d after *B. juncea* (BJ), *B. napus* (BN), or *S. alba* (SA) seed meal application at 1 (1) or 2 (2) t-ha⁻¹. No significant differences were detected for total inorganic nitrogen on 18 May. Different letters indicate significant differences for total inorganic nitrogen within sample dates ($P \leq 0.05$).

Disproportionate emergence inhibition in the *B. napus* treatments suggests that carrot germination and emergence are suppressed by relatively low concentrations of SCN⁻ or, as previously suggested (Rice et al., 2007), other phytotoxic compounds are produced from *B. napus* seed meals. Relevant to the present experiments is the fact that lettuce (*Lactuca sativa* L.) is particularly sensitive to Brassicaceae seed meal-derived allelochemicals (Rice et al., 2007). Stiehl and Bible (1989) have published an extensive list comparing species' tolerances to SCN⁻ allowing additional predictions as to which crops might be least damaged by meal amendments.

It is also possible that BSM addition may have indirectly influenced emergence by differentially stimulating bacterial and fungal populations. Our MBN results (Fig. 1) indi-

cate a substantial increase in microbial biomass in the *B. napus* treatment that may have included a variety of plant pathogens or other microorganisms that potentially produced allelopathic, phytotoxic compounds. Microbial pathogens and microbially produced allelochemicals can effectively inhibit emergence of weed and crop species (Amusa, 2006; Hoagland, 2001; McCalla and Haskins, 1964). Researchers applying low-GLS *B. napus* and high-GLS *S. alba* seed meals to soil documented increased apple root infection by resident *Pythium* spp., a pathogen known to cause pre-emergence damping-off in a variety of plant species (Mazzola et al., 2007).

Different seeding times and environmental conditions between years may provide an explanation as to why emergence inhibition was not observed in 2005. Environmental

Table 4. Apparent nitrogen (N) mineralized (ANM), change in soil total inorganic N from before seed meal application to harvest (Δ inorganic N), and plant N uptake determined at harvest for 2005 and 2006.^z

Treatment		ANM ^y	Δ soil inorganic N ^x	Plant N uptake ^w
		----- (grams N/m ²) -----		
2005				
<i>B. juncea</i>	2 t·ha ⁻¹	16.1 ab	4.8 ab	11.6
<i>B. napus</i>	2 t·ha ⁻¹	17.8 a	6.3 a	11.7
<i>S. alba</i>	2 t·ha ⁻¹	12.1 bc	1.9 b	11.1
<i>B. juncea</i>	1 t·ha ⁻¹	12.1 bc	2.9 b	9.4
<i>B. napus</i>	1 t·ha ⁻¹	11.3 bc	1.8 b	9.7
<i>S. alba</i>	1 t·ha ⁻¹	13.4 ab	2.3 b	11.2
Control		8.3 c	-2.4 c	11.0
2006				
<i>B. juncea</i>	2 t·ha ⁻¹	11.4 a	4.9 ab	7.0 ab
<i>B. napus</i>	2 t·ha ⁻¹	9.5 ab	2.8 abc	7.5 a
<i>S. alba</i>	2 t·ha ⁻¹	11.4 a	6.6 a	6.3 bc
<i>B. juncea</i>	1 t·ha ⁻¹	6.1 bc	-0.2 cd	6.4 bc
<i>B. napus</i>	1 t·ha ⁻¹	8.3 ab	1.5 bcd	6.8 abc
<i>S. alba</i>	1 t·ha ⁻¹	4.5 c	-0.1 cd	5.9 dc
Control		2.3 d	-2.6 d	5.1 d

^zWithin a column, means with different letters are significantly different ($P \leq 0.05$). Means within the same column without letters are not significantly different ($P \leq 0.05$).

^yANM = Δ soil inorganic N (pre-seed meal to harvest) + plant N uptake.

^xCombined NH_4^+ -N and NO_3^- -N, based on 0- to 50-cm depth-weighted averages.

^wCombined root and shoot N uptake at harvest.

Fig. 4. Seasonal apparent net nitrogen mineralized (ANM) expressed as a percent of total nitrogen measured (%ANM) in *B. juncea* (BJ), *B. napus* (BN), and *S. alba* (SA) seed meals amended to soil at 1 (1) or 2 (2) t·ha⁻¹ in 2005 and 2006. Calculation based on assumption that ANM measured in the control was background ANM in seed meal treatments. Error bars represent one SE of the mean.

conditions during the period between BSM application and seeding in 2005 likely supported more extensive degradation and transport of 2-propenyl ITC and SCN^- compared with 2006. During the periods between BSM application and seeding, 21 more calendar days and 969 more degree-days (0 °C base temperature) elapsed in 2005 than in 2006. Soil moisture was also higher in 2005 as a result of greater precipitation and irrigation amounts (455 mm) compared with 2006 (62 mm). Gaseous losses of 2-propenyl ITC were more probable during the longer period between meal application and planting in 2005 compared with 2006. Additionally, the greater amount of irrigation and precipitation recorded in 2005 possibly supported more extensive leaching losses of SCN^- from the

seeding zone. Leaching of SCN^- from surface-applied seed meals to depths greater than 25 cm has been observed (Hansson et al., 2008). Combined results emphasize the need to consider environmental conditions after BSM application in selecting planting dates that will minimize crop phytotoxicity.

Carrot yield. For both years, total yields (nonmarketable and marketable roots and shoots) averaged across all treatments (2005: 40 t·ha⁻¹, 2006: 35 t·ha⁻¹) were comparable to the 1993 to 2003 U.S. national average fresh market yield of 35 t·ha⁻¹ (USDA, 2007). Increased N availability in BSM treatments may have supported the higher total yields in 2006, although shoot percent N values at harvest in 2006 were similar across all treatments, including the control (Table 3), and all were within the N sufficiency range (1.5% to 2.5%) for optimum carrot growth and yields (Maynard and Hochmuth, 1997). Carrots are highly variable in their response to N fertilization (Hipp, 1978; Hochmuth et al., 1999; Rubatzky et al., 1999; Sandersen and Ivany, 1997; Westerveld et al., 2006) and tissue N concentrations have been shown to be poor estimators of carrot yields (Pettipas et al., 2006). Other essential plant nutrients (Table 1) mineralized from the BSMs may also have been important to these yield increases.

Carrot quality. We observed no differences in the percentage of nonmarketable carrots by weight or by number compared with the control, indicating that BSM treatments did not decrease carrot quality as a result of phytotoxic compound release or because of excess N. This is not surprising for 2005, because the absence of emergence inhibition in this year suggests that phytotoxic GLS degradation products released from BSMs had degraded or mobilized from the seed zone at the time of seeding. In 2006, although BSMs inhibited emergence, they

did not negatively impact carrot quality. Results from 2006 show that phytotoxic GLS degradation products can inhibit carrot emergence but do not necessarily cause longer-term plant damage. Although abundant inorganic soil N was available in BSM treatments in both years (Figs. 2B and 3B), decreased root quality often associated with excess N in the soil and plants such as forked and split roots (Bienz, 1965) was not observed. In 2005, shoot percent N in all treatments (2.74% to 3.08% N) (Table 3) was in excess (greater than 2.5%) according to established shoot percent N sufficiency guidelines for carrots at harvest (Maynard and Hochmuth, 1997), yet we observed no evidence of root damage. Additionally, we did not find any indication of increased crop damage measured at harvest from pests capitalizing on the excess N or other nutrients in BSM-amended soil.

Microbial biomass nitrogen. It is common to observe an initial increase followed by decline in MBN as we did here (Fig. 1) on addition of fresh, low C:N ratio residues under conditions favorable to microbial decomposition (Sylvia et al., 2005). Most notable is the apparent short-term inhibition of N uptake by the microbial community in the high-GLS *S. alba* and *B. juncea* treatments relative to the low-GLS *B. napus* treatment. Microbial biomass N in the *B. napus* treatment reached a maximum 4 d after meal application, whereas peak MBN in the *B. juncea* and *S. alba* treatments was delayed until at least 14 d after meal application. This is likely a result of the biocidal properties of 2-propenyl ITC and SCN^- released from *B. juncea* and *S. alba* seed meals, respectively. 2-Propenyl ITC is known to inhibit various microorganisms (Brown and Morra, 1997), including nitrifiers (Bending and Lincoln, 2000), and the toxicity of SCN^- to a variety of different microorganisms has also been reported (Beekhuis, 1975; Smith et al., 1945; van Shreven et al., 1970).

There was an apparent trend for lower MBN in the *S. alba* treatment compared with the *B. juncea* treatment for Days 4, 8, and 14, and significantly lower MBN was measured in the *S. alba* treatment on Day 8 compared with the *B. napus* and the *B. juncea* treatments (Fig. 1). The greater inhibition in *S. alba* treatments may be because SCN^- is more persistent than 2-propenyl ITC in soil (Brown et al., 1991; Morra, 2004) as previously described when discussing carrot emergence, or a specific undetermined effect of SCN^- on N cycling.

The short-term increases in MBN indicate that BSM amendments temporarily cause immobilization of soil inorganic N. Immobilization of inorganic N can be detrimental if plant N uptake needs are in excess of available soil inorganic N (Coyne, 1999). However, immobilized microbial biomass N is less susceptible to volatilization or leaching losses and thus serves as a protected source of N that can become available to plants during microbial biomass turnover (Brookes, 2001). The substantial decline in MBN between

Days 14 and 43 (Fig. 1) suggests that N immobilization was transient and that inorganic N was mineralized from the microbial community in advance of maximum plant N needs later in the growing season. Additionally, the short-term differential effects of BSM treatments on MBN did not influence longer-term N mineralization as indicated by the absence of a covariate effect ($P = 0.25$) of MBN on soil inorganic N or ANM measured 96 d after BSM application.

Soil inorganic nitrogen and apparent net nitrogen mineralized. In both years, there was a definite trend for greater apparent N mineralization in meal-amended soils than in the unamended control (Table 4) with %ANM ranging from 30% to 81% across all treatments (Fig. 4). The %ANM in all BSM treatments ranged from 55% to 81% in the 2006 96-d experimental period suggesting that N in BSMs mineralizes at a rate that is likely adequate for the N needs of many crops. It is important to recognize that these values do not necessarily represent the amount of N mineralized from organic N in the BSMs, because of the priming effect in which organic material additions stimulate mineralization of soil organic N (Kuzyakov et al., 2000). However, our results are comparable to those from previous research in which it was estimated that 60% of total seed meal-N was mineralized during a 90-d period after soil was amended with *B. napus* seed meal (Gale et al., 2006). Although we did not see a consistent yield increase caused by BSM use in short-season carrots, a crop that can require as little as 50 kg inorganic N/ha (Maynard and Hochmuth, 1997), other crops with higher N requirements may substantially benefit from a preplant application of BSMs. Additionally, a subsequent catch-crop could capitalize on the residual N, improving overall N use efficiency and maximizing agronomic benefits of BSMs when used as soil amendments.

In both 2005 and 2006, we did not observe convincing evidence that GLS types and concentrations markedly affect seasonal N mineralization. This is supported by the fact that there were no consistent differences in inorganic N concentrations or ANM in soils amended with different BSMs containing unique GLS profiles. GLS degradation products did not influence longer-term N mineralization rates as measured during either a 96- or 123-d period under irrigated conditions.

Conclusions

BSMs applied at 1 and 2 t·ha⁻¹ rates can be used to increase plant-available inorganic N in soil and improve crop yields. GLS degradation products inhibit microbial N uptake in the short term, but we did not observe convincing evidence that these compounds affect longer-term N cycling enough to inhibit plant growth or decrease N availability. Manual carrot thinning moderated the effects of emergence inhibition because no differences in harvest numbers or yields were observed. Such crop emergence inhibition

may be acceptable in a system in which seed is sown in excess and subsequently thinned, yet precautions are necessary to avoid excessive crop emergence inhibition and stand decreases. The effective use of BSMs will require knowledge of the time and environmental conditions necessary to reduce GLS hydrolysis products to a nontoxic level through microbial degradation, volatilization, or leaching. Phytotoxic considerations may add additional challenges to synchronizing N mineralization with the rate and extent of plant N uptake. Future assessment of Brassicaceae seed meals as soil amendments will require consideration of different environmental conditions, soil types, crop sensitivity to phytotoxins, time of maximum plant N uptake, and overall plant N needs.

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