

## Research Article

# Impact of Indian Mustard (*Brassica juncea*) and Flax (*Linum usitatissimum*) Seed Meal Applications on Soil Carbon, Nitrogen, and Microbial Dynamics

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There is a critical need to investigate how land application of dedicated biofuel oilseed meals affects soil ecosystems. In this study, mustard (*Brassica juncea*) and flax (*Linum usitatissimum*) seed meals and sorghum-sudangrass (*Sorghum bicolor*) were added to soil at levels of 0, 1, 2.5, and 5% (w/w). Both the type of amendment and application rate affected soil organic C, total C & N, and C & N mineralization. Mustard meal amendment initially inhibited C mineralization as compared to flax, but >50% of mustard and flax organic C was mineralized within 51 d. Nitrogen mineralization was similar for flax and mustard, except for the 2.5% rate for which a lower proportion of mustard N was converted to nitrate. The mustard meal greatly impacted microbial community composition, appearing to select for specific fungal populations. The potential varying impacts of different oilseed meals on soil ecosystems should be considered when developing recommendations for land application.

## 1. Introduction

There is currently great interest in the use of various biofuels to supplement fossil fuel supplies. One potential source of biofuels is the production of biodiesel from oilseed crops. Oilseeds, such as soybeans, have been cultivated for hundreds of years with much of the oilseed meal, or press-cake, remaining after oil extraction being used for food, animal feed, or other industrial purposes. However, the cultivation of additional oilseed varieties, at the scale necessary to provide a significant supplement to worldwide demands for fossil fuels, may saturate existing markets for these oilseed meal coproducts [1]. Additionally, in order to avoid competition between food and fuel supplies, there are growing efforts to focus upon and/or develop nonfood oilseed crops that are dedicated to the production of biofuels and other industrial products [2]. However, many of the seed meals from these non-food, dedicated biofuel crops, such as castor, contain

compounds or toxins which limit their use as food or animal feed [3–5].

One alternative use for these oilseed meals is as a soil amendment. Oilseed meals contain substantial amounts of N and varying levels of other nutrients needed for plant growth. Additionally, land application of the oilseed meals may increase levels of soil C and contribute positively to the net C effect of biofuels. Although there has been a substantial amount of research on the use of meals from some oil-producing crops as organic fertilizers, there has been a relatively limited amount of research for many of the dedicated oilseed crops [6–8].

The *Brassicaceae* family includes several oilseed plants, including Indian mustard (*Brassica juncea*), which have potential for use as dedicated oilseed crops. One unique property of many plants in the *Brassicaceae* family is that they contain glucosinolates, which when hydrolyzed are converted into biocidal chemicals such as isothiocyanates, nitriles, and

TABLE 1: Selected characteristics of Weswood loam soil.

Total N	Total C g kg <sup>-1</sup>	Organic C	Soil nutrient concentrations									Soil texture				
			P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu	Sand	Silt g kg <sup>-1</sup>	Clay	pH
0.5	12.8	5.4	34	225	5440	236	15	210	0.95	0.15	0.61	0.26	420	380	200	7.9

ionic thiocyanates [9, 10]. These compounds have been documented to have broad biocidal effects and suppress a number of soil pathogens, insects, and weeds [11–14]. Although numerous studies have documented the beneficial usage of brassicaceous plants or green tissue as biofumigants, only a limited number of studies have been conducted on use of brassicaceous oilseed meals for this purpose [11, 15–17].

Additionally, although the impacts of isothiocyanates and related compounds on microbial pathogens have been well documented, the impacts of nonpathogenic, soil microorganisms are largely unknown. In the handful of studies that have been conducted on this, it has been reported that isothiocyanates and related compounds may affect bacterial and eukaryotic community structure [18] and inhibit populations and activity of nitrifying bacteria [19]. Since alteration of soil microbial communities may directly impact C mineralization, nutrient cycling, and numerous other aspects of soil quality, additional research is needed to specifically investigate the effects of brassicaceous oilseed meals on soil microbial ecosystems.

In this study, we used seed meals of mustard and flax along with sorghum-sudangrass biomass to represent oilseed meals from the *Brassica* and non-*Brassica* families and a “common” non-oilseed residue, respectively. The major objectives of this study were to compare the effects of application of a brassicaceous oilseed meal, nonbrassicaceous oilseed meal, and lignocellulosic biomass on (1) soil C and N dynamics, including organic, mineralizable, and total fractions of C and N and (2) soil microbial communities including populations of nitrifying bacteria.

## 2. Materials and Methods

**2.1. Soil Collection and Characterization.** Weswood loam (fine-silty, mixed, superactive, thermic, Udifluventic Haplustept) was used in this study (Table 1). It is an alluvial soil in the flood plain of the Brazos River in South Central Texas. Weswood soils are well-drained loamy soils and are used as irrigated cropland [20]. Bulk soil samples were collected from 0–15 cm depth and composited. Soils were kept at field moisture and then homogenized and passed through a 2-mm sieve. Soils were tested for total C, organic C, and total N by a combustion method using an Elemental Vario Max CN analyzer (Elementar Analysensysteme, Hanau, Germany) [21–23]. Organic C was determined at 650°C while total C was determined at 950°C. Soil phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and sodium (Na) were extracted with Mehlich III solution and analyzed by inductively coupled plasma (ICP) spectrometry [24, 25]. Soil micronutrients including copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were extracted using a 0.005 M DTPA,

0.01 M CaCl<sub>2</sub>, and 0.1 M triethanolamine solution mixture and determined by ICP [26]. Soil particle size distribution was determined using the hydrometer method [27]. Soil pH was measured using a pH meter in a soil water suspension (10 g soil to 20 mL deionized water) after shaking 30 min at 180 rev min<sup>-1</sup> on a reciprocal shaker followed by standing for 30 min [28].

**2.2. Oilseed Meal and Sorghum-Sudangrass Analysis.** Mustard (*Brassica juncea*) seed meal was obtained from the *Brassica* Breeding and Research group at the University of Idaho, USA. Flax (*Linum usitatissimum*) seed meal was made by processing seeds, collected from the Texas AgriLife Research Farm in Burleson County, Tex, USA with a Komet Oil Press (Model CA59, IBG Monforts Oekotec, Mönchengladbach, Germany). Sorghum-sudangrass (*Sorghum bicolor*) biomass was collected from the Texas AgriLife Research Farm in Burleson County, Tex, USA. Seed meals were ground with a mortar and pestle and passed through a 1-mm sieve. Sorghum-sudangrass was ground with a Wiley Mill no. 4 (20 mesh screen). Organic C, total C, and total N in the biomass amendments were determined by a high temperature combustion process using an Elemental Vario Max CN analyzer (Elementar Analysensysteme) [21, 29–31]. Organic C was determined at 650°C while total C was determined at 950°C. Plant B, Ca, Cu, Fe, K, Mg, Na, P, S, and Zn were determined using a nitric acid digestion and ICP analysis [32, 33]. Residual oil content in seed meals was determined by a Minispec mq-one nuclear magnetic resonance unit (Bruker Optics Inc., Billerica, Mass, USA) fitted with a 40-mm fuse with hydrogen probe.

Glucosinolate concentrations in seed meals and biomass were determined following the International Organization for Standardization methods [34] but with a few modifications as detailed below. Samples were first defatted with one extraction and two rinses of petroleum ether by vacuum filtration. Defatted material was weighed (300 mg) into 50 mL centrifuge tubes to which 500 mg of 5-mm glass beads [35] were added and then immediately vortexed. A hot (70°C) 70% methanol : H<sub>2</sub>O solution (10 mL) was added to the samples that were then placed in a hot water bath for 20 minutes and vortexed intermittently. After which, the samples were centrifuged, and the supernatant was collected. An additional extraction was performed similar to above but with 5 mL of hot methanol rather than 10 mL. The extracts were combined, and 2 mL were added to a 0.6 mL plug of DEAE Sephadex A-25 anion exchanger and allowed to drain freely. The poly-prep chromatography columns (BioRad, Hercules, Calif, USA) were then rinsed with 1 mL deionized water and finally with two aliquots of 1 mL 0.02 M sodium acetate buffer (pH 4.5). Sulfatase solution (100 μL) was

added to the columns and allowed to sit overnight (16 hrs). Desulfoglucosinolates were eluted with 3 consecutive 1 mL volumes of deionized water. Samples were immediately separated and quantified by HPLC using a Waters 600s System Controller, 717 autosampler, and 996 photodiode array detector. The system was equipped with a Waters 3.5  $\mu\text{m}$  Symmetry Shield RP8 column (2.1  $\times$  150 mm), in which mobile phases flowed at 0.3 mL/min, and compounds were separated using an acetonitrile gradient starting at 2.0% and increasing to 95.0%. Expected retention behavior, such as time and sequence, and mass spectra were used to identify glucosinolate peaks. A calibration curve was constructed using sinigrin monohydrate (Science Lab, Houston, Tex, USA) as an external standard.

**2.3. Experimental Design.** This was a laboratory study using a completely randomized design (CRD) with factorial treatment structure. The two treatment factors were application rate and type of oilseed meals and residue. Application rates were 0.5, 1, 2.5, and 5% (w/w) (field equivalent of  $\sim$ 9, 18, 45, and 90 Mg ha<sup>-1</sup>, resp.), and added materials included mustard seed meal, flax seed meal, and sorghum-sudangrass biomass. The sorghum-sudangrass treatment was included as a lignocellulosic biomass for comparison with the oilseed meals. Each treatment had 3 replications. Blanks without meal applications were also used. In total, there were  $4 \times 3 \times 3 + 3 = 39$  experimental units, which were randomly assigned in a 25°C incubator. Two separate incubations were conducted. A microcosm study (using 400 g of soil per microcosm) was used to determine changes in organic C, total C, total N, and microbial communities over time. A second, smaller scale (50 g of soil per microcosm) was used to determine C and N mineralization rates and effects on populations of nitrifying bacteria.

**2.4. Organic C, Total C, and Total N Dynamics.** Each microcosm (a 1-liter glass jar) contained 400 g of soil adjusted to 13% (w/w) water content ( $\sim$ 40% field capacity). The various seed meal and biomass amendments were added to each microcosm and thoroughly mixed. Jars were incubated with covers loosely sealed to maintain aerobic conditions at 25°C in a controlled environment incubator. Soil moisture content was adjusted weekly by adding deionized water to the desired level according to weight. Soil was sampled from each microcosm at intervals of 3, 14, 28, and 133 d. A portion of each sample was tested for pH, and another was air-dried, ground to pass a 150- $\mu\text{m}$  sieve, and analyzed for organic C, total C, and total N as described above. An additional portion of the sample was immediately frozen and stored at  $-80^\circ\text{C}$  for subsequent microbial analyses.

**2.5. Soil C and N Mineralization.** Each microcosm (a 1-liter glass jar) contained 50 g of soil adjusted to 13% water content (w/w) along with a beaker containing 10 mL of 1.0 N NaOH. Jars were then tightly sealed and were incubated for a total duration of 51 d. Carbon mineralization rates were determined at intervals of 2, 5, 10, 16, 23, 37, 44, and 51 d using acid titration to quantify CO<sub>2</sub> evolution. After each

sampling, a new beaker with fresh 1 N NaOH solution was placed into the jar. At the completion of the incubation (51 d), a portion of the sample was immediately frozen and stored at  $-80^\circ\text{C}$  for subsequent microbial analyses. Another portion of the soil was air-dried, processed, and analyzed for organic C, total C, and total N as described above. In addition, samples were extracted with 1 M KCl (1:2) and analyzed for NH<sub>4</sub>-N colorimetrically by spectrophotometer [36] and with 1 M KCl (1:10) for NO<sub>3</sub>-N by reduction to nitrite using a cadmium column followed by determination of nitrite concentrations with a spectrophotometer [37].

**2.6. Microbial Community Composition and Dynamics.** The impacts of biomass additions on soil microbial communities were determined by total lipid-fatty acid methyl ester analysis (TL-FAME) using a hybrid lipid extraction protocol as described by Ushio et al. [38]. Briefly, samples collected 3 and 28 d after soil amendment in the C and N dynamics experiment were freeze-dried and shipped to the Ecosystem Microbiology Laboratory at the University of Wisconsin Wis, USA for lipid extraction and identification using the Microbial ID method for FAME (MIDI, Inc., Newark, Del, USA). Detected lipids containing between 10 and 20 carbons in length were used for subsequent multivariate analysis. The indicator lipids used for quantification of specific microbial groups were (1) fungi: 18:1 $\omega$ 9c, 18:2 $\omega$ 6c {MIDI Sum in Feature 19}, 18:3 $\omega$ 6c, (2) Gram+ bacteria: 15:0, a15:0, i15:0, i16:0, 17:0, a17:0, i17:0, and (3) Gram- bacteria: 16:1 $\omega$ 7c, cy17:0, cy19:0, 16:0 2OH, 16:1 2OH, 18:1 $\omega$ 7c {MIDI Sum in Feature 8} [38–41].

In addition, effects of biomass additions on populations of nitrifying bacteria were determined in the C and N mineralization experiment using a quantitative-PCR- (qPCR-) based approach. Approximately 1 g of soil from each sample was extracted using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, Calif, USA) according to the manufacturer's instructions. The DNA extracts were purified using illustra MicroSpin S-400 HR columns (GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA) and were quantified using a Quant-iT PicoGreen dsDNA assay kit (Invitrogen Corp, Carlsbad, Calif, USA).

Population levels of nitrifying bacteria were determined using primers targeting the *amoA* gene [42]. The assays were performed in a 10- $\mu\text{L}$  reaction mix containing 4.5  $\mu\text{L}$  SYBR green real master mix (5Prime, Inc., Gaithersburg, Md, USA), 0.5  $\mu\text{L}$  of each primer (200 nM), 1  $\mu\text{L}$  template (2.5 ng), 1  $\mu\text{L}$  bovine serum albumin (10 mg mL<sup>-1</sup>), and 2.5  $\mu\text{L}$  molecular-grade water (DNase-free). The qPCR conditions included an initial 10 min at 95°C followed by 40 amplification cycles of 94°C for 60 s, 60°C for 60 s, and 72°C for 60 s. Each run in the analysis included a set of standards, positive and negative controls, and samples (all including three analytical replicates) on a 96-well plate. Melting curve analysis of the qPCR products was performed after each assay to confirm qPCR amplification quality. The qPCR was performed using an Eppendorf Mastercycler ep realplex thermal cycler (Eppendorf, Hamburg, Germany).

Standards for qPCR were generated by PCR amplifying the *amoA* gene from the genomic DNA of *Nitrosomonas*

TABLE 2: Nutrient concentrations of flax and mustard oilseed meals and sorghum-sudangrass biomass.

Biomass type	C	N	C:N	Oil <sup>a</sup>	P	K	Ca	Mg	Na	S	Zn	Fe	Cu	Mn	B
	g kg <sup>-1</sup>				g kg <sup>-1</sup>						mg kg <sup>-1</sup>				
Flax	493	59	8.4	157	6.6	19	3.1	5.4	1.3	3.3	44	32	15	46	30
Mustard	478	61	7.8	162	11.0	19	4.9	5.6	1.3	16.0	62	44	12	47	16
Sorghum	430	18	23.9	nd <sup>b</sup>	1.2	20	7.1	1.1	1.3	1.9	47	303	9	56	<9

<sup>a</sup> Residual oil content in seed meals following oil extraction.

<sup>b</sup> nd: not determined.

*europaea* [43]. The PCR products were confirmed on an agarose gel and then cloned into a pGEM-T Easy vector following the manufacturer's instructions (Promega, Madison, Wis, USA). Positive clones were isolated and extracted for plasmid DNA using a Wizard SV Miniprep kit (Promega). Plasmid DNA concentrations were quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen) and were used for preparing appropriate dilution standards for the qPCR assays. The plasmid DNA concentrations ranging from  $5.0 \times 10^{-3}$  to  $5.0 \times 10^{-7}$  ng  $\mu\text{L}^{-1}$  DNA were used to generate the qPCR standard curves and to quantify the absolute *amoA* gene copy numbers per gram of soil.

**2.7. Statistical Analyses.** Statistical analyses were conducted using the R statistical programming language v2.10.1 [44] and the Rcmdr package v1.6.0 [45]. Analysis of variance (ANOVA) was used to evaluate the effects of biomass amendment, rate, and/or time on soil and microbial community characteristics. Two-way and repeated measures ANOVA were used, where appropriate, and in cases where interactive effects were significant, the data sets were broken down into simpler subsets and analyzed using one-way ANOVA. Where possible, post hoc evaluations of means were conducted using Tukey tests. Data that did not meet assumptions of normality or homogeneity of variance were log-transformed prior to analysis. All results are presented as nontransformed values, and results were considered to be statistically significant when  $P \leq 0.05$ . Additionally, nonmetric multidimensional scaling of the soil microbial communities, based upon FAME composition, was carried out using the Bray-Curtis similarity metric in the PAST software package, version 2.03 [46].

### 3. Results

**3.1. Soil Characteristics.** Weswood soils were slightly alkaline with a pH of 7.9 (Table 1). Soil total C was  $12.8 \text{ g kg}^{-1}$ , of which  $5.4 \text{ g kg}^{-1}$  was organic C. Soils also contained various concentrations of extractable macro- and micronutrients. Phosphorus was in the moderate category, while K, Ca, Mg, and S were rated as high in availability according to Texas AgriLife Extension Guidelines. Iron, Zn, Mn, and Cu were low.

**3.2. Composition of Seed Meals and Sorghum-Sudangrass Biomass.** A major difference between oilseed meals and sorghum-sudangrass biomass was their N concentrations and

hence C:N ratios (Table 2). The mustard seed meal used in this experiment contained  $478 \text{ g kg}^{-1}$  C and  $61 \text{ g kg}^{-1}$  N with a C/N ratio of 7.8. Similarly, flax seed meals had  $493 \text{ g kg}^{-1}$  C and  $59 \text{ g kg}^{-1}$  N with a C/N ratio of 8.4. Sorghum-sudangrass had a much lower N concentration ( $18 \text{ g kg}^{-1}$ ) and a much higher C/N ratio (24). Mustard seed meal contained about 5 times as much S as flax seed meal and more than 8 times that of sorghum-sudangrass. Both seed meals also had much higher concentrations of P, Mg, and B, while sorghum-sudangrass had substantially more Fe. Concentrations of other elements, such as K, Na, Zn, and Mn were comparable in all three amendments. Residual oil contents were similar in mustard and flax seed meals, both being about  $160 \text{ g kg}^{-1}$ . The mustard seed meal contained several glucosinolate-related compounds with the dominant being 2-propenyl glucosinolate (sinigrin) at a concentration of  $157.9 (\pm 15.1) \mu\text{mol g}^{-1}$ . Neither the flax seed meal nor the sorghum-sudangrass residue contained detectable levels of glucosinolates.

**3.3. Organic C, Total C, and Total N Dynamics.** A significant portion of the added oilseed meal organic C was degraded rapidly following addition to soil, with organic C losses slowing after few weeks (Figure 1). For example, about 21, 21, 33, and 37% of organic C in the 0.5, 1, 2.5, and 5% flax treatments, respectively, were lost within the first two weeks. After 4 months of incubation, approximately 48, 57, 67, and 72% of added organic C had been removed in the 0.5, 1, 2.5, and 5% flax treatments resulting in soil organic C values of 6.7, 7.5, 9.5, and  $12.3 \text{ g kg}^{-1}$ , respectively. Degradation of organic C was similar in the mustard-amended treatments with approximately 30, 61, 70, and 79% of added organic C removed after 4 months in the 0.5, 1, 2.5, and 5% mustard treatments, respectively. The loss of organic C in sorghum-sudangrass treatments was smaller relative to the seed meal treatments. About 4, 18, 14, and 17% of added organic C in the 0.5, 1, 2.5, and 5% sorghum-sudangrass treatments, respectively, was lost within two weeks. By the end of the 4-month incubation, only 21 to 44% of added organic C had been degraded resulting in soil organic C levels of 6.9, 8.8, 11.6, and  $17.5 \text{ g kg}^{-1}$  in the 0.5, 1, 2.5, and 5% sorghum-sudangrass treatments, respectively, which were greater than their oilseed treatment counterparts. There were no significant changes among the control samples throughout the incubation. Soil total C dynamics, which included organic and carbonate C, followed a pattern similar to organic C (Figure 2).

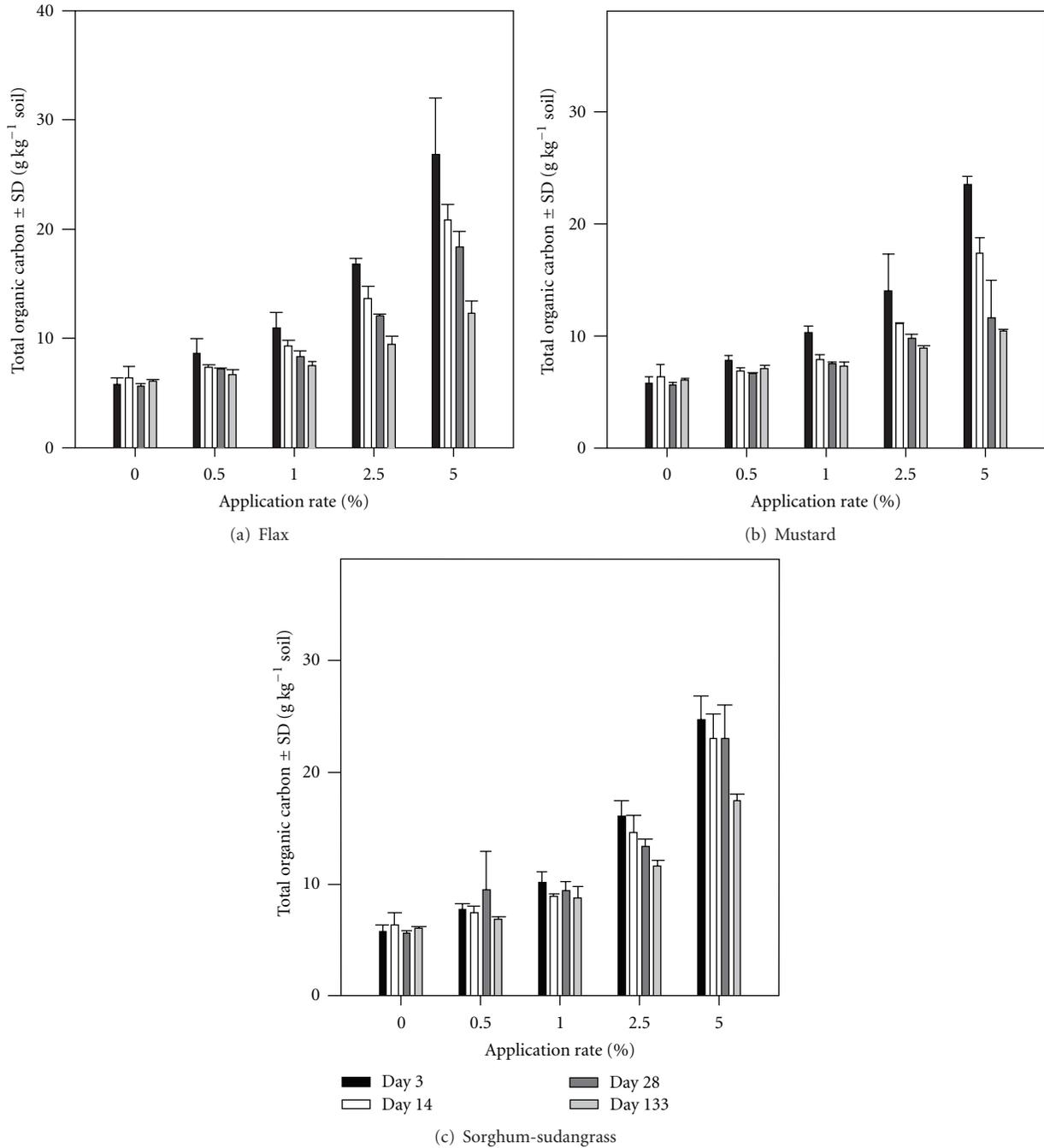


FIGURE 1: Soil organic C dynamics as affected by added oilseed meals and sorghum-sudangrass biomass after 3, 14, 28, and 133 d of addition. Means are based on 3 replications. Bars represent  $\pm$  standard deviation of the mean.

Biomass addition significantly enhanced soil total N levels with flax and mustard seed meals having larger effects than the sorghum-sudangrass biomass (Figure 3). In contrast to the soil C dynamics, soil total N did not exhibit significant changes over the incubation period.

Application of oilseed meals and sorghum-sudangrass had different impacts on soil pH. The initial soil pH was 7.9. After 4 months of incubation, soil pH decreased to 6.8 and 6.9 with 5% mustard and flax seed meal additions, respec-

tively. The pH reduction was less in treatments receiving 0.5 and 1% amendments, usually within 0.5 unit of the original soil pH. There was no change in pH change among sorghum-sudangrass treatments.

**3.4. Carbon Mineralization.** Both the type of biomass amendment and application rate had significant effects on soil C mineralization rates (Figure 4). Regardless of amendment type, C mineralization increased with increasing application.

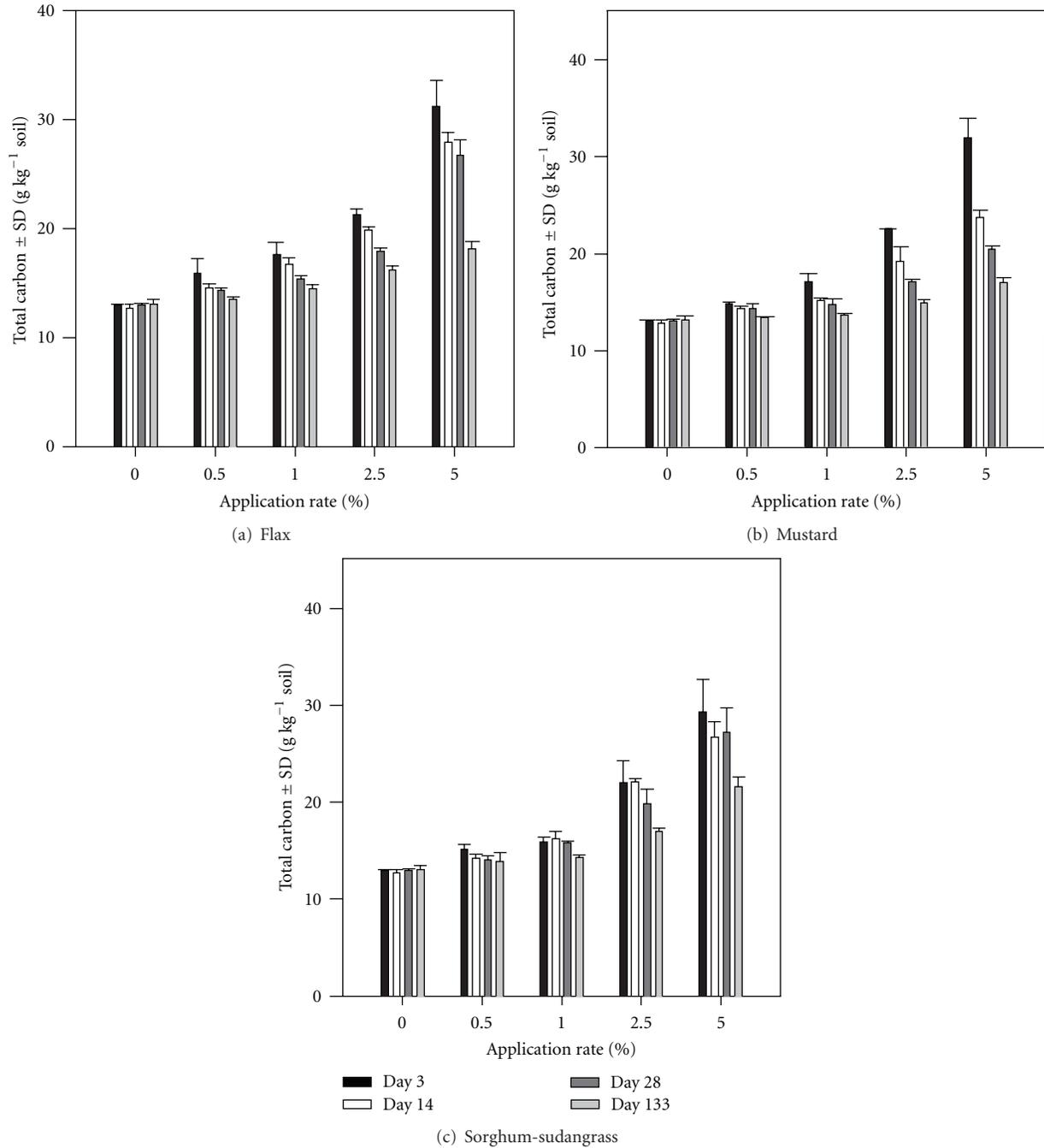


FIGURE 2: Soil total C dynamics as affected by added oilseed meals and sorghum-sudangrass biomass after 3, 14, 28, and 133 d of addition. Means are based on 3 replications. Bars represent  $\pm$  standard deviation of the mean.

Interestingly, flax and mustard seed meals and sorghum-sudangrass biomass each had distinct C mineralization patterns over the incubation period. For flax, the CO<sub>2</sub> flux peaked shortly (~2–5 d) after flax seed meal addition and was followed by a rapid decrease. By the end of the third week, the CO<sub>2</sub> flux had generally stabilized, except that addition treatments of 2.5 and 5% were greater than controls, 0.5, and 1% rates. Similarly for sorghum-sudangrass, soil CO<sub>2</sub> flux showed a very sharp initial increase following the addition

of biomass, decreased precipitously within 2 weeks, and thereafter maintained a relatively stable rate which was still higher than the controls, especially at higher application rates. In contrast, the soil CO<sub>2</sub> peak flux following addition of mustard did not occur until around 10 d of incubation for most addition rates and was followed by a more gradual decrease to levels approaching the unamended soil. Data for the 5% amendment rate for all biomass types is not shown due to several early (2–5 d) CO<sub>2</sub> flux values that exceeded

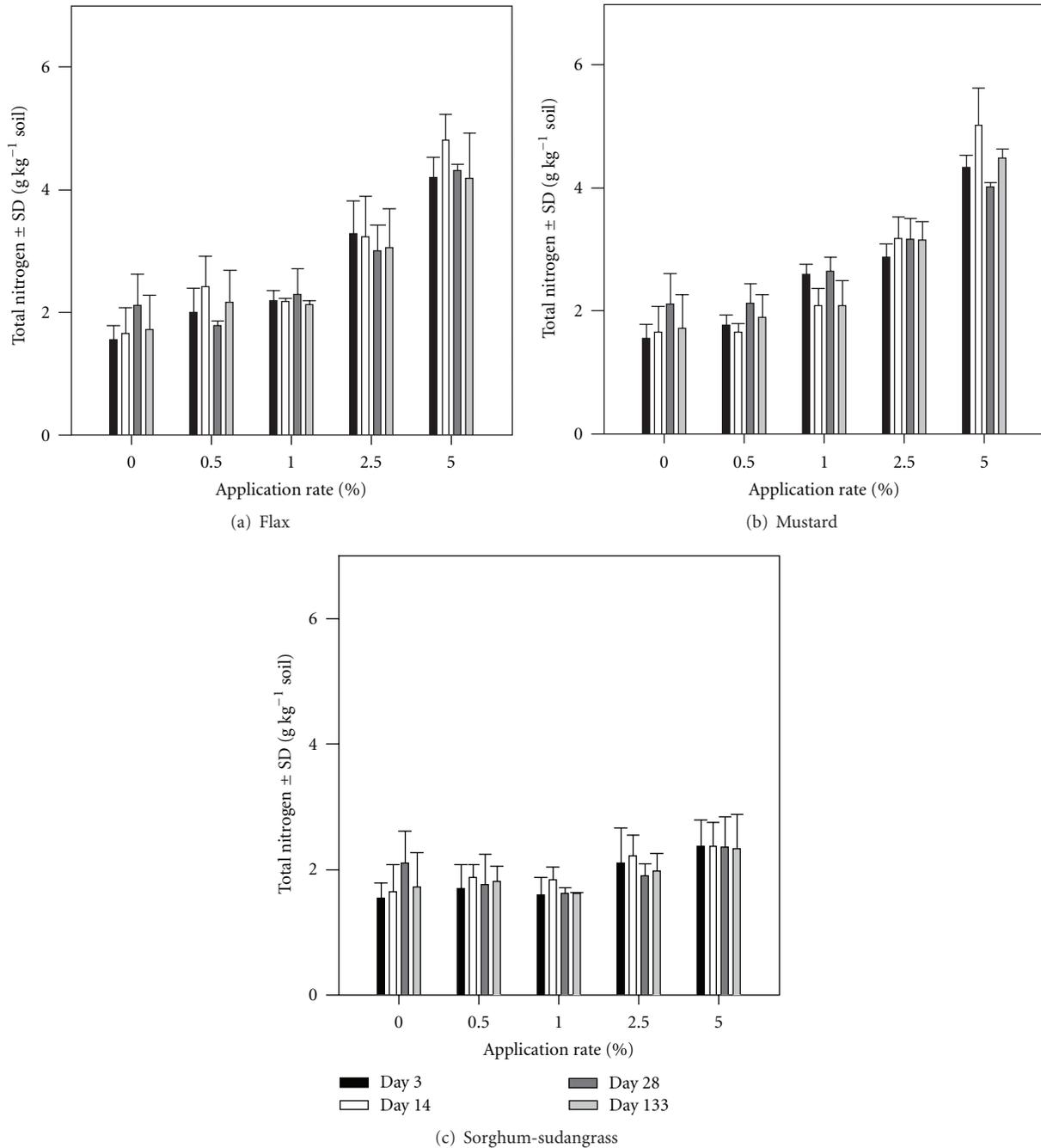


FIGURE 3: Soil total N dynamics as affected by added oilseed meals and sorghum-sudangrass biomass after 3, 14, 28, and 133 d of addition. Means are based on 3 replications. Bars represent  $\pm$  standard deviation of the mean.

detection limits; however, the 5% amendment results generally trended with those for the 2.5% amendment rate.

Cumulative C mineralization and removal of organic C during the 51 d of incubation followed the order of mustard  $\approx$  flax > sorghum-sudangrass (Table 3). After 51 d, approximately 25 to 53% of added organic C remained in the mustard- and flax-amended soil in contrast to the sorghum-sudangrass-amended soil for which 60 to 71% of the added organic C remained.

**3.5. Nitrogen Mineralization.** Similar to the C mineralization results, treatments receiving flax and mustard meals resulted in much higher amounts of mineralized N than treatments receiving sorghum-sudangrass biomass (Table 3). After application of oilseed meals, the levels of soil inorganic N increased dramatically in proportion to application rate, reaching maximum levels of 705 and 857 mg kg<sup>-1</sup> at the 5% amendment rate. In contrast, soil inorganic N levels were much lower in the sorghum-sudangrass-amended soils

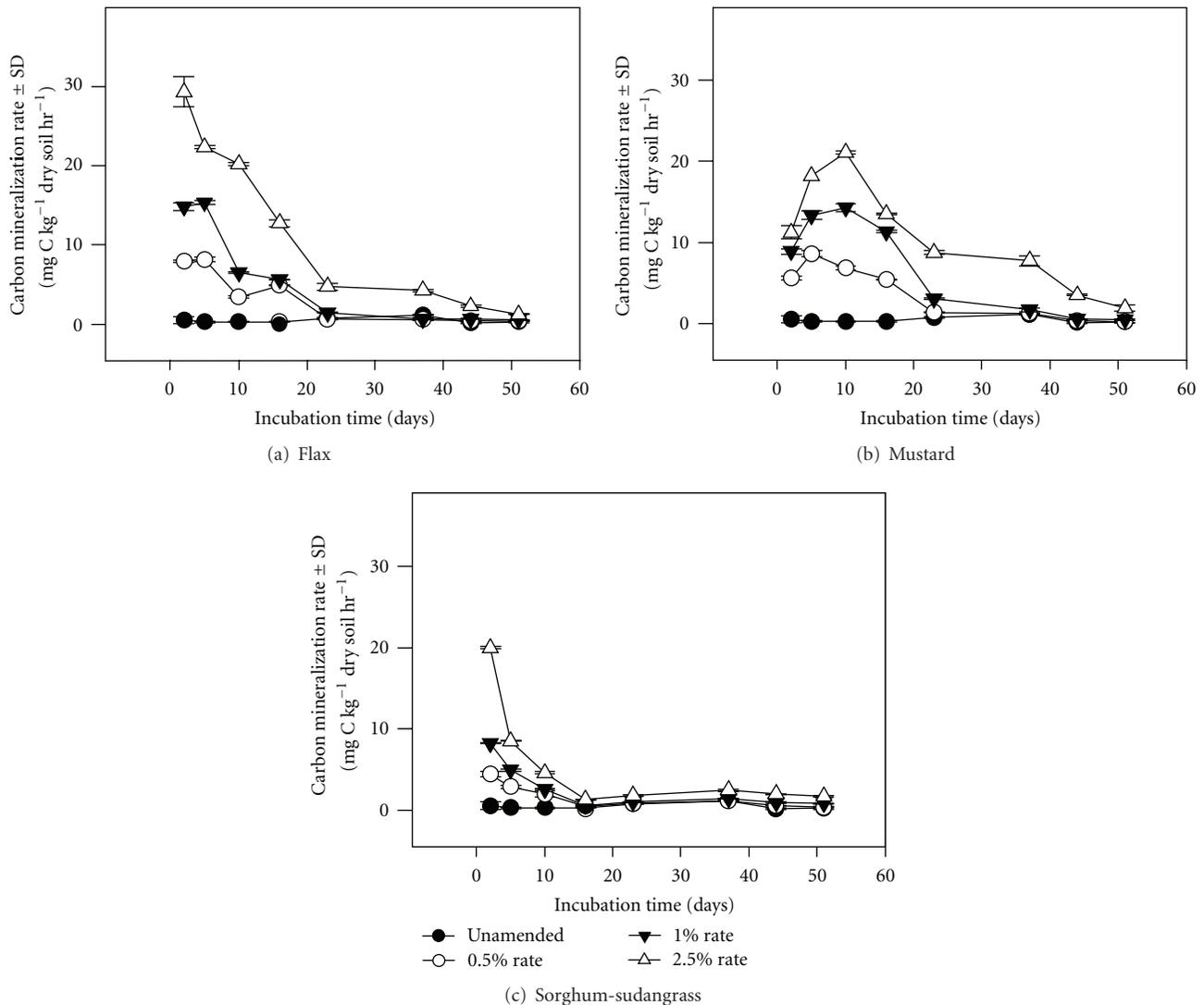


FIGURE 4: Soil C mineralization dynamics as affected by added oilseed meals and sorghum-sudangrass biomass with time. (●: unamended; ○: 0.5%; ▼: 1%; △: 2.5% application rate). Means are based on 3 replications. Bars represent  $\pm$  standard deviation of the mean. Error bars are hidden when smaller than the symbols.

(7.8 to 26.6 mg kg<sup>-1</sup>) and tended to decrease at higher application rates. Approximately 14–30% of total N in the mustard- and flax-amended soils was inorganic N after 51 d in comparison to only 4.4% in the unamended soil and 0.5 to 3.2% in the sorghum-sudangrass-amended soil.

The amount of the soil inorganic N existing as NH<sub>4</sub>-N or NO<sub>3</sub>-N varied depending upon both the rate and type of biomass amendment. For the sorghum-sudangrass treatments, the majority of mineralized N at all amendment rates was NH<sub>4</sub>-N (Table 3). In contrast, for flax and mustard treatments, the primary form (>90%) of inorganic N at the end of incubation for the lower rates of amendment (0.5 and 1%) was NO<sub>3</sub>-N. At the highest amendment rate (5%), essentially all (>99%) of the inorganic soil N was NH<sub>4</sub>-N. For the mustard and flax oilseed meals, the levels of soil NH<sub>4</sub>-N and NO<sub>3</sub>-N were similar for most application rates except

for the 2.5% application rate for which mustard resulted in much higher levels of NH<sub>4</sub>-N (297 v. 24 mg kg<sup>-1</sup>) and lower levels NO<sub>3</sub>-N (243 v. 601 mg kg<sup>-1</sup>) than did the flax treatments.

**3.6. Microbial Community Composition and Dynamics.** Both the type and rate of biomass addition affected the abundance of specific microbial groups and the overall composition of the soil microbial community. Within 3 d, levels of soil microbial indicator lipids had increased approximately 2- to 10-fold in amended soils (Table 4). At each application rate, the increase was greatest in the mustard-amended soils. As illustrated by the fungal:bacterial ratios, this was largely a result of increased fungal biomass in the mustard-amended soils in contrast to the flax and sorghum-sudangrass where there was less of a fungal impact. Generally, after 28 d,

TABLE 3: Soil carbon and nitrogen concentrations 51 d after amendment with various rates of flax or mustard oilseed meals or sorghum-sudangrass biomass.

Amendment rate (%)	Biomass type	Soil nutrient concentrations					
		Total C (g kg <sup>-1</sup> soil)	Organic C (g kg <sup>-1</sup> soil)	Added organic C remaining (%)	Total N (g kg <sup>-1</sup> soil)	NH <sub>4</sub> -N (mg kg <sup>-1</sup> soil)	NO <sub>3</sub> -N (mg kg <sup>-1</sup> soil)
0.5	Unamended	12.95 <sup>a</sup>	5.77 <sup>a</sup>	—	0.90 <sup>a</sup>	2.33 <sup>a</sup>	37.67 <sup>a</sup>
	Flax	13.84 <sup>b</sup>	6.57 <sup>b</sup>	45.68 <sup>a</sup>	1.06 <sup>b</sup>	6.93 <sup>ab</sup>	147.20 <sup>b</sup>
	Mustard	13.33 <sup>ab</sup>	6.70 <sup>b</sup>	53.36 <sup>a</sup>	1.13 <sup>b</sup>	10.82 <sup>ab</sup>	161.83 <sup>b</sup>
	Sorghum-sudangrass	13.60 <sup>ab</sup>	6.96 <sup>b</sup>	71.13 <sup>a</sup>	0.82 <sup>a</sup>	16.82 <sup>b</sup>	9.73 <sup>c</sup>
1.0	Unamended	12.95 <sup>a</sup>	5.77 <sup>a</sup>	—	0.90 <sup>a</sup>	2.33 <sup>a</sup>	37.67 <sup>a</sup>
	Flax	14.42 <sup>ab</sup>	7.76 <sup>bc</sup>	47.12 <sup>ab</sup>	1.33 <sup>b</sup>	11.26 <sup>b</sup>	239.17 <sup>b</sup>
	Mustard	13.68 <sup>a</sup>	7.04 <sup>b</sup>	33.71 <sup>a</sup>	1.36 <sup>b</sup>	13.68 <sup>b</sup>	278.27 <sup>b</sup>
	Sorghum-sudangrass	16.52 <sup>b</sup>	8.00 <sup>c</sup>	60.20 <sup>b</sup>	1.15 <sup>ab</sup>	23.02 <sup>c</sup>	3.50 <sup>c</sup>
2.5	Unamended	12.95 <sup>a</sup>	5.77 <sup>a</sup>	—	0.90 <sup>a</sup>	2.33 <sup>a</sup>	37.67 <sup>a</sup>
	Flax	17.22 <sup>b</sup>	9.76 <sup>b</sup>	35.18 <sup>a</sup>	2.07 <sup>b</sup>	23.66 <sup>b</sup>	601.17 <sup>b</sup>
	Mustard	15.34 <sup>c</sup>	8.42 <sup>c</sup>	25.26 <sup>a</sup>	1.90 <sup>b</sup>	296.58 <sup>c</sup>	243.30 <sup>c</sup>
	Sorghum-sudangrass	19.24 <sup>d</sup>	21.60 <sup>d</sup>	67.44 <sup>b</sup>	1.16 <sup>c</sup>	5.52 <sup>a</sup>	3.03 <sup>d</sup>
5.0	Unamended	12.95 <sup>a</sup>	5.77 <sup>a</sup>	—	0.90 <sup>a</sup>	2.33 <sup>a</sup>	37.67 <sup>a</sup>
	Flax	23.30 <sup>b</sup>	16.57 <sup>bc</sup>	45.36 <sup>a</sup>	2.72 <sup>b</sup>	701.66 <sup>b</sup>	2.97 <sup>b</sup>
	Mustard	22.25 <sup>b</sup>	14.99 <sup>b</sup>	40.52 <sup>a</sup>	2.98 <sup>b</sup>	853.64 <sup>b</sup>	3.73 <sup>b</sup>
	Sorghum-sudangrass	25.18 <sup>b</sup>	19.34 <sup>c</sup>	65.45 <sup>b</sup>	1.46 <sup>a</sup>	4.93 <sup>c</sup>	2.83 <sup>b</sup>

Means within a column, for a given amendment rate (including the unamended control), followed by the same letter(s) are not significantly different at  $P < 0.05$ .

total levels of microbial indicator lipids remained higher in amended soils, but the fungal:bacterial ratios were not significantly different from the unamended soil.

All of the biomass types impacted the overall soil microbial community composition within 3 d of amendment (Figure 5). However, the mustard and sorghum-sudangrass had the greatest impacts. Interestingly, the microbial community in the mustard-amended soil was much more similar to the sorghum-sudangrass-amended soil than that of the flax-amended soil. After 28 d, all of the amended treatments remained different than the unamended soil; however, they were generally more similar to the unamended soil than they were after 3 d.

Likewise, both the rate and type of biomass amendment impacted levels of nitrifying bacteria in the C & N mineralization experiment. After 51 d, application of oilseed meals increased the levels of nitrifying bacteria between 7- and 74-fold, at the 0.5, 1, and 2.5% amendment rates, as compared to the unamended soil (Figure 6). However, neither flax nor mustard oilseed meal increased populations of nitrifying bacteria at the 5% amendment rate. Levels of nitrifying bacteria for sorghum-sudangrass-amended soil, at all application rates, were not different from the unamended control.

#### 4. Discussion

4.1. Soil C Dynamics. Carbon added with the oilseed meals and sorghum-sudangrass exhibited different mineralization patterns in soil. Organic matter in the oilseed meals showed

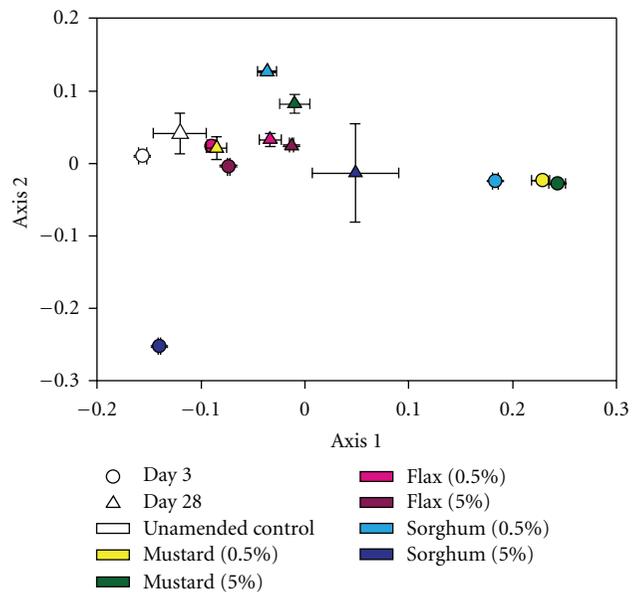


FIGURE 5: Nonmetric multidimensional scaling analysis of soil microbial communities, based upon total lipid-fatty acid methyl ester profiles, 3 and 28 d after amendment of soil with 0.5 or 5% rates of flax or mustard oilseed meals or sorghum-sudangrass biomass. Means are based on 3 replications. Bars represent  $\pm$  standard error of the mean.

extensive mineralization, particularly shortly after application. Carbon mineralization, however, was greatly reduced

TABLE 4: Microbial lipid biomarkers in soil 3 and 28 days after amendment with various rates of flax or mustard seed meal or sorghum-sudangrass biomass.

Time (days)	Amendment rate (%)	Biomass type	TL-FAME <sup>1</sup> markers (nmol g <sup>-1</sup> soil)					
			Total bacteria	Gram + bacteria	Gram – bacteria	Fungi	Total <sup>2</sup>	Fungal : bacterial ratio
3	0	Unamended	15.86 <sup>a</sup>	7.64 <sup>a</sup>	8.22 <sup>a</sup>	9.30 <sup>a</sup>	25.16 <sup>a</sup>	0.59 <sup>a</sup>
	0.5	Flax	30.21 <sup>bc</sup>	15.54 <sup>b</sup>	14.68 <sup>a</sup>	23.19 <sup>b</sup>	53.40 <sup>b</sup>	0.77 <sup>a</sup>
	0.5	Mustard	25.83 <sup>b</sup>	18.29 <sup>b</sup>	7.54 <sup>b</sup>	113.36 <sup>c</sup>	139.20 <sup>c</sup>	4.44 <sup>b</sup>
	0.5	Sorghum-sudangrass	33.69 <sup>c</sup>	25.02 <sup>c</sup>	8.67 <sup>a</sup>	86.84 <sup>d</sup>	120.53 <sup>d</sup>	2.57 <sup>c</sup>
3	0	Unamended	15.86 <sup>a</sup>	7.64 <sup>a</sup>	8.22 <sup>a</sup>	9.30 <sup>a</sup>	25.16 <sup>a</sup>	0.59 <sup>a</sup>
	5.0	Flax	57.80 <sup>b</sup>	35.54 <sup>b</sup>	22.25 <sup>b</sup>	40.95 <sup>b</sup>	98.74 <sup>b</sup>	0.71 <sup>b</sup>
	5.0	Mustard	42.82 <sup>c</sup>	24.00 <sup>c</sup>	18.82 <sup>bc</sup>	206.10 <sup>c</sup>	248.91 <sup>c</sup>	4.80 <sup>c</sup>
	5.0	Sorghum-sudangrass	56.37 <sup>b</sup>	42.79 <sup>b</sup>	13.58 <sup>ac</sup>	2.31 <sup>d</sup>	58.69 <sup>d</sup>	0.04 <sup>d</sup>
28	0	Unamended	16.66 <sup>a</sup>	8.21 <sup>a</sup>	8.44 <sup>a</sup>	16.09 <sup>a</sup>	32.75 <sup>a</sup>	0.97 <sup>a</sup>
	0.5	Flax	34.45 <sup>b</sup>	18.05 <sup>b</sup>	16.40 <sup>b</sup>	41.92 <sup>ab</sup>	76.38 <sup>ab</sup>	1.21 <sup>ab</sup>
	0.5	Mustard	45.19 <sup>b</sup>	25.96 <sup>c</sup>	19.23 <sup>b</sup>	34.42 <sup>ab</sup>	79.61 <sup>ab</sup>	0.76 <sup>a</sup>
	0.5	Sorghum-sudangrass	33.74 <sup>b</sup>	22.42 <sup>bc</sup>	11.32 <sup>a</sup>	73.04 <sup>b</sup>	106.78 <sup>b</sup>	2.51 <sup>b</sup>
28	0	Unamended	16.66 <sup>a</sup>	8.21 <sup>a</sup>	8.44 <sup>a</sup>	16.09 <sup>a</sup>	32.75 <sup>a</sup>	0.97 <sup>a</sup>
	5.0	Flax	83.66 <sup>bc</sup>	49.14 <sup>b</sup>	34.52 <sup>b</sup>	99.58 <sup>a</sup>	183.24 <sup>b</sup>	1.19 <sup>a</sup>
	5.0	Mustard	120.34 <sup>b</sup>	97.62 <sup>c</sup>	22.72 <sup>ab</sup>	202.59 <sup>b</sup>	322.92 <sup>c</sup>	1.69 <sup>a</sup>
	5.0	Sorghum-sudangrass	81.67 <sup>c</sup>	59.58 <sup>b</sup>	22.10 <sup>ab</sup>	103.26 <sup>a</sup>	184.93 <sup>b</sup>	1.34 <sup>a</sup>

Means within a column, for a specific amendment rate (including the unamended control) and day, followed by the same letter(s) are not significantly different at  $P < 0.05$ .

<sup>1</sup>TL-FAME: total lipid-fatty acid methyl ester analysis.

<sup>2</sup>Total TL-FAME marker represents the sum of Gram-positive bacteria, Gram-negative bacteria, and fungi for a given community.

after 2 to 3 wks. The C compounds were apparently more labile in the oilseed meals than the sorghum-sudangrass since the application of the oilseed meals resulted in more rapid and greater cumulative C mineralization. This is not surprising since typical crop residues, such as sorghum-sudangrass, contain large amounts of lignin and lower amounts of protein [47], while oilseed meals usually contain relatively large amounts of protein and fatty acids but less than 20% fiber [48]. The lower C:N ratios in oilseed meals also likely helped to maintain higher C mineralization rates relative to the sorghum-sudangrass.

In addition to differences in the rate and extent of C mineralization for the oilseed meals as compared to the sorghum-sudangrass biomass, there were also differences between the mustard and flax oilseed meals. For flax, the C mineralization peaked very quickly (within 2 d) while it was delayed until around 10 d in the mustard-amended soil. In addition, the initial CO<sub>2</sub> flux from 5% mustard seed meal addition was smaller than that from 1 and 2.5% additions. These results are consistent with those of Snyder et al. [8] who found that a 2% amendment of mustard seed meal (*B. juncea*) delayed the peak and magnitude of C mineralization as compared to the same amount of rapeseed (*B. napus*) containing a much lower glucosinolate content. Thus, in both studies, this initial reduction in C mineralization was likely due to the high level of glucosinolate compounds in the mustard [10, 49]. However, these secondary chemicals are susceptible to microbial degradation and have

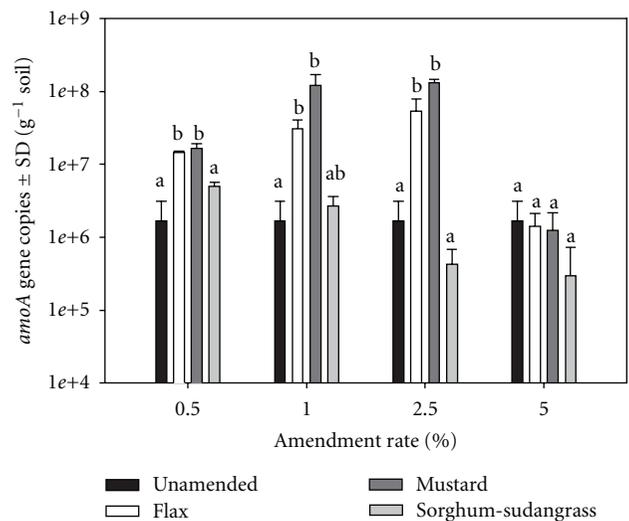


FIGURE 6: Populations of nitrifying bacteria (expressed as copies of *amoA*) 51 d after amendment of soil with flax or mustard oilseed meals or sorghum-sudangrass biomass. Means are based on 3 replications. Bars represent  $\pm$  standard deviation of the mean. Means with different letters for the same application rate are significantly different ( $P < 0.05$ ).

a relatively short half-life once applied to soils, often being degraded within two weeks [18, 50, 51]. This is consistent with our results in that the CO<sub>2</sub> flux peaked after the likely

degradation of the biocidal compounds in mustard treatments. It is also possible that after the initial effect, the soil microbial communities adapted to the chemicals and selected for microorganisms that were more resistant to the biocidal compounds which then flourished to produce the peak in CO<sub>2</sub> flux. Although C mineralization in the mustard-amended soil was delayed at the beginning of the experiment, by the end of the experiment the cumulative C mineralization was equal to that in the flax-amended soil.

At the end of the 4-month incubation, soil total C increased from 12.8 to 14.5 g kg<sup>-1</sup> in the 1% flax meal treatment, amounting to an addition of about  $5 \times 10^3$  kg of C ha<sup>-1</sup> on a field basis (assuming the same mineralization rate, soil bulk density of 1.2 g cm<sup>-3</sup>, and biomass applied to a depth of 0–15 cm). This result has two implications. First, once land-applied, not all of the C contained in the meals can be counted as sequestered C. A large portion, over half in this study, is likely to be mineralized quickly and released into the atmosphere. Second, the remaining C, however, may have the potential for temporary or longer-term C sequestration thus enhancing the “C neutrality” of any resulting biofuels.

The reduction in soil pH for oilseed meal-amended treatments presumably occurred due to the production of organic and/or inorganic acids during organic matter degradation. Although this could be a concern if applying high rates of oilseed meals to acidic soils, application rates would likely be below 18 Mg ha<sup>-1</sup> (1%) under typical field conditions and result in relatively small (<0.5 unit) decreases in pH, depending upon the soil. A reduction in pH could be beneficial for alkaline soils; however, this might result in the release of inorganic soil carbon and contribute negatively to the carbon budget of the produced biofuel. Although we did not see evidence of large losses of soil C due to pH reduction in this experiment, it should be considered in subsequent experiments on alkaline soils.

**4.2. Soil N Dynamics and Nitrifying Bacteria.** While considerable C was lost through respiration, total N remained in the soil under our experimental conditions, as was demonstrated by nearly constant values in all treatments throughout the incubation period. However, a very different scenario might occur under field conditions where there is greater potential for soil N losses through denitrification, runoff, and/or leaching.

Given that the oilseed meals had much higher N content and lower C:N ratios than the sorghum-sudangrass biomass, it was not surprising that they resulted in a greater amount of mineralized N. Under the same assumptions as previously stated for C, there would be about 400 kg mineralized N ha<sup>-1</sup> for a 1% rate of flax or mustard meal addition in the field. This indicates that oilseed meals have great potential as a slow-release N fertilizer [7]. In contrast, adding sorghum-sudangrass actually resulted in significant N immobilization.

Generally, after 51 d of incubation, there was no difference in the amount of ammonification and nitrification in the mustard- and flax-amended soils, for a given level of amendment rate, with essentially all inorganic N being either NO<sub>3</sub>-N (0.5 and 1% amendments) or NH<sub>4</sub>-N (5%

amendment) for both seed meal types. However, there were significant differences in nitrification at the 2.5% rate of amendment. After 51 d of incubation, essentially all inorganic N in the flax treatment was NO<sub>3</sub>-N while approximately 55% of the inorganic N in the mustard treatment was NH<sub>4</sub>-N. Others have demonstrated that isothiocyanates (ITC) can inhibit soil nitrifying bacteria through direct reductions in their populations and activities, resulting in nitrification being inhibited by 35–65% [19]; however, in that study, NH<sub>4</sub>-N was added to the soil concurrent with the ITCs. It is possible that allelochemicals released from the mustard oilseed meal, added at high rates, may have inhibited nitrification in the current experiment; however, we found no significant differences in the population sizes of nitrifying bacteria in the mustard- and flax-amended soils, for a given application rate. Another possible explanation is a reduction in ammonification. For oilseed meals, the organic N would first have to be converted to NH<sub>4</sub>-N before it could be nitrified. A recent study in which a 2% rate of mustard meal (*B. juncea*) was applied to soil found a reduction in mineralization of N and levels of NH<sub>4</sub>-N and NO<sub>3</sub>-N as compared to the same rate of rapeseed (*B. napus*) containing a much lower glucosinolate content [8]; however, levels of both NH<sub>4</sub>-N and NO<sub>3</sub>-N in the mustard-amended soil were essentially equivalent to the those in the rapeseed-amended soil by 30 to 45 d. Since we did not detect differences in the numbers of nitrifying bacteria in the 2.5% mustard and flax treatments, it is likely that other processes such as delayed or reduced ammonification contributed at least partially to the lower levels of NO<sub>3</sub>-N after 51 d. It is possible that, although the biocidal chemicals in the mustard did not appear to impact population sizes of nitrifying bacteria, that they did impact their metabolic activity and thus decreased levels of nitrification; however, the abundance of NH<sub>4</sub>-N and very low values of NO<sub>3</sub>-N in both the 5% mustard and 5% flax treatments indicates that additional processes, such as high levels of ammonia or volatile sulfur compounds, were also likely contributing to low nitrification activities at high levels of seed meal amendment [52–54].

**4.3. Soil Microbial Communities.** Not surprisingly, each of the added biomass types impacted the soil microbial communities as compared to the unamended control. However, it was somewhat surprising that the mustard generally selected for a microbial community that was more similar to the sorghum-sudangrass than to the flax oilseed meal. This appeared to be largely due to greater fungal biomass in the mustard and sorghum-sudangrass treatments. For the mustard, this is especially interesting given the number of studies documenting its adverse effects on various soil fungi [11, 15, 16]. Our initial presumption was that the mustard would broadly inhibit soil fungal populations. The initial suppression of C mineralization in the mustard treatments tended to agree with this. However, it appears that the mustard selected for a population(s) of fungi that were resistant to the presence of any biocidal products produced from the glucosinolates in the mustard. Only a handful of studies have investigated the impacts of isothiocyanates and related compounds on soil microbial communities [18, 55, 56]. Even though

these studies have focused upon the impact of ITCs added as pure chemicals, in harvested green tissue, or in living plants instead of being from seed meals, they all have generally indicated that glucosinolate breakdown products (e.g., ITCs) can at least transiently impact microbial communities, especially fungi, at high concentrations. In the current study, the microbial communities in all amended treatments had recovered to compositions more resembling the unamended soil after 28 d, but it is unclear if these changes might have longer-term impacts on other soil processes. Ongoing, DNA-based analyses are being used to further characterize the impacts of these seed meals on the soil microbial communities.

## 5. Conclusion

Oilseed meals are different from traditional soil additives such as crop residues in that they contain greater amounts of N and easily decomposable C. Three aspects require special attention when considering land application of oilseed meals. First, a large proportion of C in oilseed meals will be rather quickly respired and released into the atmosphere as CO<sub>2</sub>. Carbon dynamics in subsequent months and years following application will determine how much C is eventually incorporated into soil organic matter and more information regarding these long-term effects is needed in order to develop a more complete picture of C cycling with seed meals. Second, oilseed meals contain large amounts of N, which like C, will be mineralized once in soil. On the one hand, N mineralization from oilseed meals can provide N needed for crop growth and should reduce N fertilizer requirements. On the other hand, large amounts of available N may result in environmental degradation. Therefore, site-specific field experiments are needed to determine the release rate and fate of N from oilseed meals, which can then be used to develop best management practices regarding N. Third, many oilseed meals from dedicated biofuel crops, such as mustard, may release allelochemicals which can affect soil microorganisms. Mustard seed meals at higher rates demonstrated inhibitory effects on C mineralization at the beginning of incubation and also dramatically altered the soil microbial communities. However, this effect appeared to be transient, and soil biological activities soon recovered either due to the degradation of the allelochemicals and/or soil microbial adaptation. Further research is needed on the longer-term effects of these oilseed meals on soil C dynamics and soil ecosystems in order to develop specific recommendations for land application of these biofuel co-products.

## Acknowledgments

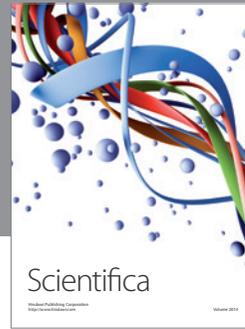
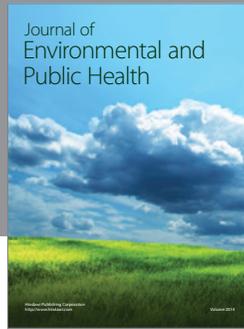
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