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Potential of meadowfoam (*Limnanthes alba*) seed meal as an organic source of nitrogen

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ABSTRACT

Meadowfoam (*Limnanthes alba* Hartw. Ex Genth.), a member of the Brassicales order, is grown for its oil, which generates seed meal as a by-product. The potential of meadowfoam seed meal (MSM) for agronomic purposes is relatively unexplored, although MSM has shown promise for weed and pest control. Because of its high protein content, MSM may also be useful as an organic N fertilizer. The objective of this research was to characterize the dynamics of C and N released from MSM during its decomposition. The potential of MSM to produce plant-available N was evaluated in a laboratory incubation using rates similar to those applied in field studies of its weed control potential. Respiration (CO_2 production rate), the activities of two enzymes involved in the degradation of MSM ($\beta_{1,4}$-glucosidase and leucine aminopeptidase), and inorganic N (NH_4^+ and NO_3^-) were measured during a 56-d period. Respiration peaked during the first week, indicating rapid MSM decomposition, which was coincident with maximum potential activity of leucine aminopeptidase. Inorganic N (dominantly NH_4^+) peaked later, at two weeks, when about half of the N in the added MSM had been mineralized. Nitrification was delayed until six weeks, presumably because of inhibitory effects of the MSM, a phenomenon observed with seed meals of other brassicaceous plants. These results demonstrate the potential use of MSM as a slow-release, organic N amendment in crop production systems.

1. Introduction

Several plant species within the Brassicales order are cultivated for oil production, with the oil used in cooking, for industrial purposes, or as a biofuel feed stock (Blackshaw et al., 2011; McVetty et al., 2016). After the expression of the oil from the seeds, a seed meal byproduct remains. A unique feature of seed meal obtained from Brassicales species is that many contain bioactive glucosinolates and related molecules, which are used as biopesticides and bioherbicides (Brown and Morra, 1997). In addition, the seed meal is relatively high in protein, and thus nitrogen (N), and has been used as animal feed or as a soil fertility amendment (Shamsi et al., 2012).

Meadowfoam (*Limnanthes alba* Hartw. Ex Benth.) is a species in the Brassicales order, specifically of the Limnanthaceae family. It is native to southern Oregon and northern California, and is well adapted to poorly drained soils of the Willamette Valley, where it is used as a winter rotation crop (Ehrensing et al., 1997; Steiner et al., 2006). The seeds of meadowfoam are harvested for the production of oil rich in long-chain fatty acids (20:1, 22:1, and 22:2), which has a variety of industrial uses (Knapp and Crane, 1995). The pulp remaining after oil extraction (meadowfoam seed meal, hereafter referred to as MSM) contains 25% protein, 22% fiber, and 4% glucosinolates (Purdy and Craig, 1987). Its glucosinolate content and composition make it useful for suppression of weeds (Vaughn et al. 1996; Stevens et al., 2009; Intanon et al., 2014, 2015a) and soil-borne pests (Bartelt and Mikolajczak, 1989; Zasada et al., 2012; Şimşek Erşahin et al., 2014). The fiber and protein content of MSM make it suitable as an animal feed (Throckmorton et al., 1981; Miller and Cheeke, 1986) or potentially as an organic soil amendment.

Intanon et al. (2015a) tested the potential of MSM as an herbicide and fertility amendment in several field experiments. In one year, MSM added to a Chehalis silty clay loam at 3, 5, and 7% (w/w, 1.22–2.86 kg m^{-2}) reduced weeds, increased the yield and N content of lettuce (the test plant), and left higher concentrations of residual nitrate (NO_3^-) at harvest. A follow-up study the next year at the same site but with a Malabon silty clay loam did not find a yield response to either 7% MSM or an equivalent amount of urea-N (168 kg N ha^{-1}), although lettuce N content was significantly higher. At weekly intervals during the first 28 days of this second field study, Intanon et al. (2015b) measured several indicators of the microbial community and its

Abbreviations: BG, $\beta_{1,4}$-glucosidase; LAP, leucine aminopeptidase; MSM, meadowfoam seed meal

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activity. They found that MSM stimulated soil respiration and increased soil microbial biomass several fold. Potential activities of $\beta_{1,4}$-glucosidase (BG) and $\beta_{1,4}$-N-acetylglucoaminidase were higher in MSM treatments but phosphatase activity was less affected. Community-level physiological profiles, measured using Biolog EcoPlates, also responded to MSM amendment, suggesting a shift in the metabolic potential of the microbial community. Collectively, the studies of Intanon et al. (2015a,b) suggested that soil microbial activity responds positively to the addition of MSM; however, the specific response of N—cycling processes was not evaluated.

The effect on N cycling of seed meals from several other, glucosinolate-containing Brassicales species (e.g., *Brassica napus*, *B. juncea*, *B. carinata*, *Sinapis alba*) has been studied (Takahashi et al., 2004; Balesh et al., 2005; Snyder et al., 2010). Because these seed meals are high in N, with C:N ratios usually less than 10, N is rapidly mineralized as they are decomposed. There is sometimes a delay in nitrification (Takahashi et al., 2004; Moore et al., 2010; Snyder et al., 2010), which has been attributed to inhibitory effects of glucosinolates contained in the seed meal (Bending and Lincoln, 2000); however, this inhibition has not always been observed (Strauss et al., 2014; Marchetti et al., 2015).

Our goal was to investigate the microbial and enzymatic processes underlying the use of MSM as a N fertilizer (Intanon et al., 2015a,b). In particular, we examined the temporal dynamics of potential enzyme activities, net N mineralization, and nitrification associated with the release of N during MSM decomposition. Based on prior studies with other glucosinolate-containing seed meals, we expected that nitrification would be inhibited, at least temporarily.

2. Material and methods

2.1. Soil and plant materials

An incubation experiment was set up to evaluate the decomposition of MSM and concurrent release of N during its decomposition. A Malabon silty clay loam soil was collected from the Lewis-Brown Horticulture Research Farm, Oregon State University, near Corvallis, Oregon, USA (43°33′N, 123°12′W). This soil is classified as a Pachic Ultic Argixeroll (Soil Survey Staff, 2010). Soil was collected from the top 20 cm, air-dried, sieved through a 2-mm sieve, and preserved in closed containers until used. The soil had a pH of 6.5, organic C content of 11.7 ± 0.3 mg C g^{-1} and organic N content of 0.72 ± 0.00 mg N g^{-1}.

Meadowfoam seed meal was obtained from Natural Plant Products, Inc. (Salem, OR, USA). The MSM (407 ± 4 mg C g^{-1}, 44.8 ± 0.3 mg N g^{-1}) was ground in a coffee mill and passed through a 1-mm sieve before use (Intanon et al., 2015b).

2.2. Incubation experiment

Soil was wet up to 0.22 kg H_2O kg^{-1} soil (~50% water-filled pore space) with deionized water and incubated in the dark for a week at 24 °C to restore biological activity and to allow the flush of respiration to return to baseline rates. Three treatments were established with MSM added and thoroughly mixed into the soil at rates of 0 (control), 1% (w/w), and 3% (w/w). Soil (20 g dry weight) from these treatments was weighed into 50-ml polypropylene tubes, 24 tubes for each treatment. Six uncapped tubes of each treatment were randomly assigned to 1-l, glass canning jars, yielding four replicate jars per treatment. Jars were covered with polyethylene film (permeable to air but not water vapor) and incubated in the dark at 24 °C. This set up allowed for easy removal of soil for destructive sampling at six times during the incubation.

2.3. Microbial activity measurements

As a general indicator of MSM degradation, respiration was measured on days 1, 2, 4, 7, 14, 21, 28, 42, and 56 by removing the polyethylene film and capping the jars with a gas-tight lid equipped with a septum. The concentration of CO_2 in the headspace of the jars was measured using a Picarro G2101 CO_2 analyzer (Picarro Inc., Dr., Santa Clara, CA, USA), with sampling done shortly after jars were capped and again 1–3 h later, depending upon the rate of CO_2 production.

Net N mineralization and nitrification were calculated from changes in inorganic N (NH_4^+ and NO_3^-) during the incubation. Following each respiration measurement, one tube of soil was removed from each jar on days 7, 14, 21, 28, 42, and 56. Soil (10 g) was extracted by shaking in 40 ml 2 M KCl for 1 h, filtered (Whatman 42), and concentrations of NH_4^+ and NO_3^- were determined colorimetrically (Hood-Nowotny et al., 2010) using a GEN5 microplate reader (BioTek, Winooski, VT, USA). Remaining soils were stored frozen (−20 °C) for subsequent enzyme assays.

Potential activities of BG and leucine aminopeptidase (LAP) were measured using standard fluorimetric enzyme assays (German et al., 2011), with 4-MUB-N-acetyl-b-glucosaminide as the substrate for BG and L-leucine 7-amido-4-methylcoumarin as the substrate for LAP. Soil suspensions were prepared by adding 1 g of soil to 100 ml of 50 mM sodium acetate buffer (pH 5.0), dispensed into 96-well microplates, incubated at 25 °C for 2 h (BG) or 24 h (LAP), and measured using a GEN5 microplate reader (BioTek, Winooski, VT, USA).

2.4. Statistical analysis

Because the 24 tubes of each treatment were randomly assigned to four replicate jars for the incubation and tubes were removed at random for analysis, we considered each measurement as independent. Thus, we used a two-way ANOVA to examine the effects of treatment and sampling time. The interaction term (treatment-by-time) was significant for all response variables measured. Because our primary interest was the effect of the treatment rather than temporal changes, we used Tukey's HSD test to determine the effect of treatment at each sampling time. Cumulative CO_2 production from MSM was modeled using single exponential kinetics, which fit all replicates with $r^2 > 0.983$. Data are presented as means and standard errors (n = 4). All statistical analyses were done with SigmaPlot 13.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Soil respiration

Addition of MSM rapidly stimulated microbial activity, with respiration responding immediately and reaching a maximum rate 2 days after addition (Fig. 1). During the first week of the incubation, respiration was significantly greater in soils receiving MSM; thereafter rates were similar (Fig. 1). Cumulative respiration at the end of the incubation was 0.19 mg C g^{-1} soil for the non-amended control, and 1.18 mg C g^{-1} soil for the 1% and 5.76 mg C g^{-1} soil for the 3% MSM treatments. This represents a loss of 24% of the added C for the 1% and a 47% loss for the 3% rates of MSM addition, assuming that there was no priming of soil organic matter.

3.2. Soil enzyme activity

The potential activity of BG, an indicator of cellulose degradation, varied little during the 56-day incubation for the non-amended control and 1% MSM treatment, which were not significantly different at any sampling time (Fig. 2A). At the 3% amendment level, potential BG activity was significantly higher than the control and 1% MSM treatments at every time point, beginning at about 220 nmol g^{-1} soil h^{-1}, increasing to 550 nmol g^{-1} soil h^{-1}, and declining to 300 nmol g^{-1} soil h^{-1} by the end of the incubation (Fig. 2A).

The potential activity of LAP, an indicator of protein degradation,