AN ABSTRACT OF THE DISSERTATION OF

Suphannika Intanon for the degree of Doctor of Philosophy in Crop Science presented on September 26, 2013.

Title: Bioherbicide Use of Plant Defensive Compounds in Meadowfoam Seed Meal

Abstract approved:

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Carol A. Mallory-Smith

Meadowfoam (*Limnanthes alba* Hartw. ex Benth) seed meal (MSM), a by-product of meadowfoam oil extraction, has a plant defensive compound known as glucosinolate glucolimnanthin (GLN). Myrosinase enzymes present in soil microbes and meadowfoam seeds can convert GLN to glucosinolate breakdown products (GBPs), which demonstrate herbicidal activity and have the potential to be used as bioherbicides. The goals of this research were to evaluate the effectiveness of MSM on weed control and to explore the optimal timing, rate, and application method for further use of MSM as a bioherbicide. Adding active myrosinase from freshly ground meadowfoam seeds to MSM increased its phytotoxicity. In a greenhouse study, no lettuce emergence was observed for six days in soil amended with 3% by weight activated MSM. In a field application, MSM provided a nitrogen source and promoted lettuce growth when lettuce seedlings were transplanted seven days after MSM incorporation. Co-occurrence of herbicide and fertilizer effects was observed with all

MSM concentrations. MSM concentrations of 5% and 7% provided greater weed emergence suppression than the 3% concentration but no difference in weed biomass was observed between MSM concentrations. A split MSM application resulted in a significant benefit for weed control, similar to a single MSM application; however for the split application, the concentration and time should be adjusted to prevent residual crop injury. Activated MSM inhibited spiny sowthistle (*Sonchus asper* (L.) Hill) greater than 95% for emergence and 80% for biomass compared to the untreated control. Soil microbes reallocated carbon input from MSM application to biomass and enzyme production. The reallocation occurred quickly, within 7 to 14 days, after MSM application. Microbial biomass increased by at least 85% for carbon and 95% for nitrogen with MSM application compared to the untreated control. β-*N*-acetylglucosaminidase activity was highly correlated with microbial biomass nitrogen and was involved in the acquisition of nitrogen from organic sources. Isothiocyanate showed potent herbicidal activity and was detected only in activated MSM. 3-Methoxyphenylacetic acid (MPAA), a previously unidentified GBP with herbicidal activity, was discovered in soil amended with non-activated and activated MSM. A single MSM application at 2.86 kg $m^{-2}$ as a pre-emergent soil amendment benefited crop yield, weed suppression, and soil carbon and nitrogen inputs.

# Bioherbicide Use of Plant Defensive Compounds in Meadowfoam Seed Meal

by
Suphannika Intanon

A DISSERTATION

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degree of

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APPROVED:

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Major Professor, representing Crop Science

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Head of the Department of Crop and Soil Science

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Suphannika Intanon, Author

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# DEDICATION

To my grandfathers, Chankeaw Intanon and Jantip Nontathum, and friends, Tipwan Sankamwinich and Elena Sanchez.

# BIOHERBICIDE USE OF PLANT DEFENSIVE COMPOUNDS IN MEADOWFOAM SEED MEAL

## CHAPTER 1

## GENERAL INTRODUCTION

### Meadowfoam

Meadowfoam (*Limnanthes alba* Hartw. ex Benth.) is an industrial oil seed crop in the Limnanthaceae family, order Brassicales, which is native to southern Oregon and northern California (Kleiman 1990). Meadowfoam is a winter rotation crop in perennial grass seed production cropping systems in the Willamette Valley of Oregon (Ehrensing et al. 1997; Steiner et al. 2006). Grass seed growers currently face significant problems with grass weed control because crops and weeds are similar in life history traits and biological and physiological responses. Meadowfoam is one broadleaf rotational crop that can benefit grass seed growers because herbicides for grass control can be used on the crop. Meadowfoam can be grown and harvested using the equipment and infrastructure that growers already have in place for grass seed and grain crops (Ehrensing et al. 1997). After harvesting, the small amount of meadowfoam straw remaining as residue is easily pulverized and requires no field burning (Ehrensing et al. 1997; Purdy and Craig 1987).

Unlike most other broadleaf crops, meadowfoam can grow in many types of soil and is well adapted to the poorly drained soils of the Willamette Valley (Ehrensing et al. 1997). The Oregon State Agricultural Experiment Station began research and development of meadowfoam in 1967. Commercial development began in 1980 (Purdy and Craig 1987). Meadowfoam oil is 98% unsaturated and is rich in unique long-chain

20:1, 22:1, and 22:2 fatty acids (Knapp and Crane 1995). The oil extracted from meadowfoam seed possesses a high oxidative stability that makes it less likely to go rancid under high temperature or in the presence of oxygen. Meadowfoam oil is useful for a wide range of products including cosmetics, lubricants, rubber additives, plastics, and biodiesel (Burg and Kleiman 1991; Hirsinger 1989; Steiner et al. 2006).

**Meadowfoam Seed Meal**

Seed meal, a by-product of the seed oil extraction process, consists of the seed and the seed coat. The seed meal is commonly used for animal nutrition and agricultural soil organic amendments to composts or manures. Seed meals of some Brassicaceae species are not desirable for animal feeding due to the deleterious health effects of high glucosinolate contents (Tripathi and Mishra 2007). Brassicaceae seed meals, for example, brown mustard (*Brassica juncea* L.) (Handiseni et al. 2011; Rice et al. 2007) and yellow mustard (*Sinapis alba* L.) (Handiseni et al. 2011; Hansson et al. 2008) with high glucosinolate content have pre-emergent herbicidal activity on various weed species.

Meadowfoam seed meal (MSM) is a by-product of meadowfoam oil extraction. It is about 70% of the biomass of harvested seed and at present has limited commercial uses. MSM has 25% protein, 22% fiber, and 4% glucosinolates (Purdy and Craig 1987). Finding additional uses for the seed meal would make the crop more economically attractive to produce.

MSM has some characteristics that suggest its potential utility in agriculture as a soil amendment to enhance plant growth (Linderman et al. 2007), suppress weeds (Machado 2007; Stevens et al. 2009; Vaughn et al. 1996; Vaughn et al. 2006), and inhibit

soil pests such as nematodes (Zasada et al. 2012), and insects (Bartelt and Mikolajczak 1989). MSM has been shown to have the potential to act as a bioherbicide on downy brome (*Bromus tectorum* L.) (Stevens et al. 2009; Machado 2007), and velvetleaf (*Abutilon theophrasti* Medik.) (Vaughn et al. 1996).

The effectiveness of MSM depends on its concentration. Low levels of MSM may be a growth stimulant for vegetable crops (Vaughn et al. 2008). MSM at concentrations of 1% to 2% by volume amended with a peat-based soil-less medium stimulated seedling growth of conifer species (Linderman et al. 2007). At MSM concentrations of greater than 2% by volume or weight, the herbicidal effect was observed on the inhibition of seed germination (Linderman et al. 2007; Machado 2007; Stevens et al. 2009; Vaughn et al. 1996; Vaughn et al. 2006). Laboratory and greenhouse studies have confirmed the herbicidal effect of MSM on seeding emergence and growth compared to the untreated control (Machado 2007; Stevens et al. 2009; Vaughn et al. 1996).

**Glucosinolates and Phytotoxicity**

Glucosinolates are a group of plant secondary metabolites in which glucosinolate and/or its hydrolysis products are biologically active (Vaughn 1999) with fungicidal, bacteriocidal, nematocidal, allelopathic, and cancer chemoprotective properties (Fahey et al. 2001). More than 120 glucosinolates have been identified within 16 dicotyledonous families, primarily in Brassicaceae. Because meadowfoam is closely related to Brassicaceae (being in the same order of Brassicales), meadowfoam, like other mustard plants, contains the glucosinolate known as glucosinolate glucolimnanthin (GLN).

Glucosinolate breakdown products occur when a cell containing glucosinolate is ruptured and the myrosinase enzyme is present (VanEtten and Tookey 1978). The enzyme and water facilitate the hydrolysis process (Fenwick and Heaney 1982). Myrosinase is present in the seed (Borek et al. 1996; Fenwick et al. 1982; Tani et al. 1974; Thangstad et al. 1991) and is produced by some soil microbes (Gimsing and Kirkegaard 2009; Rakariyatham et al. 2005; Sakorn et al. 2002; Tani et al. 1974). Glucosinolates can be hydrolysed enzymatically to form isothiocynate, nitrile, and thiocyanate (Rosa et al. 1997).

The herbicidal effects in Brassicaceae and non-Brassicaceae seed meals have been attributed to glucosinolate breakdown products. Isothiocyanate has the most potent phytotoxicity for pest control (Bartelt and Mikolajczak 1989; Brown and Morra 1996; Morra and Kirkegaard 2002; Vaughn et al. 2006; Zasada et al. 2012) but has a short-half life and fast degradation (Borek et al. 1995). Nitrile has been reported to have herbicidal properties in MSM (Vaughn et al. 1996; Stevens et al. 2009). Nitrile can be formed during autolysis and under acidic condition (pH 3). It can also be induced by heat and steam during the oil extraction process (Rosa et al. 1997). Thiocyanate has been shown to exhibit herbicidal properties in yellow mustard meal and is produced at pH 4 to 7 (Borek and Morra 2005).

GLN comprises between 2 to 4% of MSM (Purdy and Craig 1987). With the presence of myrosinase, GLN is hydrolized to glucosinolate breakdown products (GBPs) which 3-methoxybenzyl isothiocyanate (isothiocyanate) and 3-methoxyphenelacetonitrile (nitrile) have been detected in relatively greater amounts than other GBPs (Figure 1.1) (Stevens et al. 2009). However, enzymatic hydrolysis in MSM is rare because myrosinase

in MSM is denatured during the oil extraction with hot hexane (Linderman et al. 2007). Activation by adding myrosinase enzyme from freshly ground meadowfoam seeds can result in the quantitative conversion of GLN to GBPs (Stevens et al. 2009). To preserve the bioactivity of glucosinolates, the appropriate way to apply a glucosinolate-containing seedmeal is soil incorporation (Gimsing and Kirkegaard 2009; Mathiessen and Kirkegaard 2006).

**Weed Management in Organic Farming and the Experimental Approach**

Weed control options are limited in organic farming systems. Integration of cultural practices and mechanical methods can be an effective strategy for organic farmers. Reliance on intensive mechanical weed control results in soil compaction, breakdown of soil structure, a shift in microbial activity, and loss of organic matter (Martens and Martens 2002). Hand weeding is time consuming, costly, and labor intensive. Alternative options that can enhance crop growth and suppress weed pressure in organic farming are of public interest.

Developing the use of MSM as a bioherbicide could benefit both meadowfoam growers and organic farmers; however, more information is required on application methods. Therefore, the goal of this study was to evaluate the effectiveness of MSM on weed control and to explore the optimal timing, rate, and application method for further use in farming systems. In addition, the effects of organic compounds from MSM on soil microbial composition and function were observed. We conducted our studies on multiple levels (Figure 1.2).

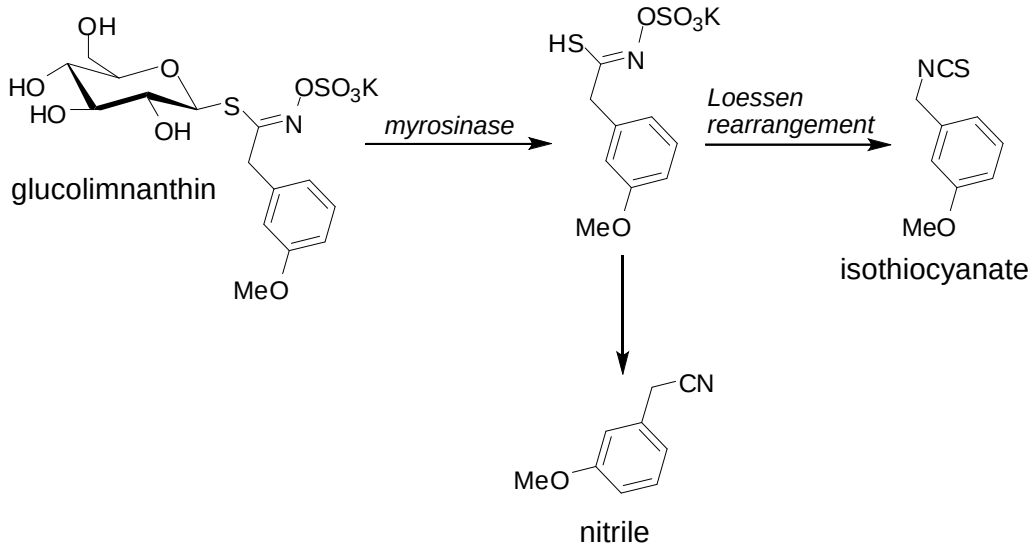



Figure 1.1. The production of prominent glucolimnanthin breakdown products in meadowfoam seed meal.

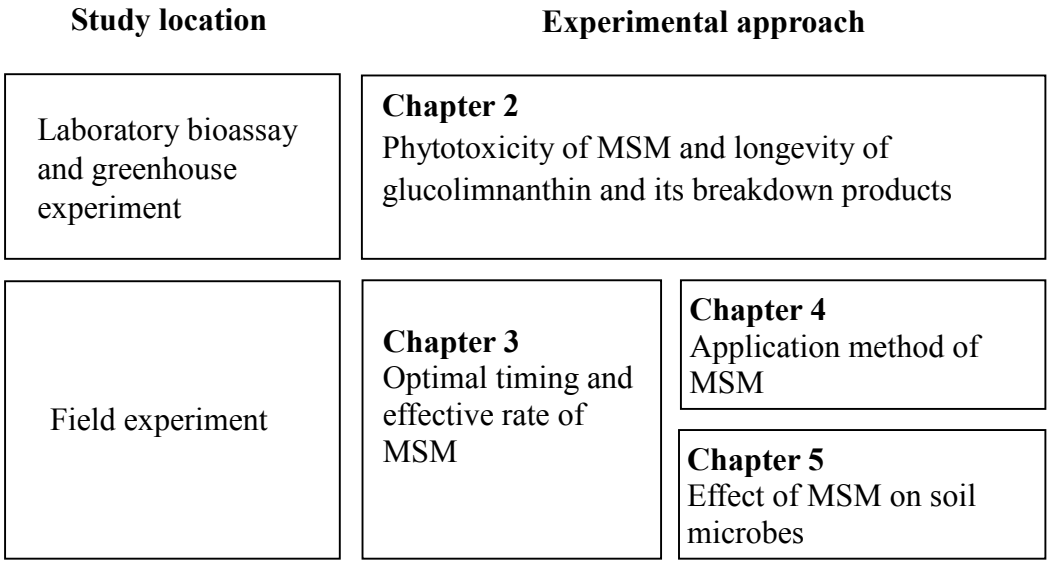



Figure 1.2. The structure of the dissertation, including study site and experimental approach. MSM = meadowfoam seed meal.

# CHAPTER 2

# MEADOWFOAM SEED MEAL AS A SOIL AMENDMENT WITH PRE-EMERGENT HERBICIDAL ACTIVITY

## ABSTRACT

Meadowfoam (*Limnanthes alba* Hartw. ex Benth.) is an oilseed crop grown in western Oregon. After oil extraction, meadowfoam seed meal (MSM), a by-product, is 70% of the harvested crop yield. MSM has 2 to 4% concentration of a plant secondary metabolite, glucosinolate glucolimnanthin (GLN). Myrosinase enzymes present in soil microbes and meadowfoam seeds convert GLN to glucosinolate breakdown products (GBPs), which have herbicidal activity and potential use as bioherbicides. Studies were conducted to compare the effect of MSM to other Brassicaceae seed meals, to evaluate the use of MSM as a soil amendment, and to determine the fate and persistence of GLN and GBPs in soil and their effects on the germination and growth of lettuce. The activation process was performed by adding 1% by weight of freshly ground meadowfoam seed to MSM. Results from the growth chamber experiments indicated that activated meadowfoam and brown mustard (*Brassica juncea* L.) seed meals provided the most suppression of lettuce germination and growth. Activated MSM applied as a soil amendment inhibited lettuce germination and growth in a greenhouse experiment. No lettuce emergence was observed for six days in soil amended with activated MSM. GLN and GBP analyses were conducted for 18 days after seed meal incorporation. In activated MSM, GLN was converted into 3-methoxybenzyl isothiocyanate (isothiocyanate), within 24 hours. By day three, the isothiocyanate was degraded. 3-Methoxyphenylacetonitrile (nitrile), a thermal breakdown product of GLN found in MSM, persisted for at least 12

days. 3-Methoxyphenylacetic acid (MPAA), a previously unknown metabolite of GLN, appeared at day three. Its identity was confirmed by LC-UV and high resolution LC-MS/MS comparisons with a standard MPAA. Soil incubation with commercial nitrile confirmed nitrile as a parent compound of MPAA. All GBPs inhibited lettuce germination with isothiocyanate about 6 and 13 times more effective than MPAA and nitrile, respectively. Biodegradation of GLN and GBPs in the soil suggests that MSM has potential uses as a pre-emergence bioherbicide.

# INTRODUCTION

Meadowfoam (*Limnanthes alba* Hartw. ex Benth.) is an industrial oil seed crop. It is a winter annual crop in the Limnanthaceae family, order Brassicales, which is native to southern Oregon and northern Califonia (Kleiman 1990). Meadowfoam is well adapted to poorly drained soils and is a winter rotation crop in the Willamette Valley of Oregon (Ehrensing et al. 1997; Steiner et al. 2006). Meadowfoam oil is 98% unsaturated and is rich in unique long-chain 20:1, 22:1, and 22:2 fatty acids (Knapp and Crane 1995). The oil extracted from meadowfoam seed possesses a unique oxidative stability that makes it useful for a wide range of products including cosmetics, lubricants, rubber additives, plastics, or biodiesel (Burg and Kleiman 1991; Hirsinger 1989; Steiner et al. 2006).

About 70% of the biomass of harvested seed remains following meadowfoam oil extraction. This by-product, known as meadowfoam seed meal (MSM), has limited commercial uses. Finding additional uses for the seed meal would make the crop more economically attractive to produce. Meadowfoam seed meal has some characteristics that suggest its potential utility in agriculture as a soil amendment to enhance plant growth (Linderman et al. 2007), suppress weeds (Machado 2007; Stevens et al. 2009; Vaughn et al. 1996; Vaughn et al. 2006), and inhibit soil pests such as nematodes (Zasada et al. 2012), and insects (Bartelt and Mikolajczak 1989).

A plant secondary metabolite in MSM is a glucolimnanthin (GLN), the major glucosinolate of meadowfoam. MSM has 2 to 4% contents of GLN (Purdy and Craig 1987). Glucolimnanthin is one of 120 identified glucosinolates within 16 families of dicotyledonous plants (Fahey et al. 2001). Glucosinolate structures consist of *β*-

thioglucoside *N*-hydroxysulfates with a side chain and a sulfur-linked *β*-D-glucopyranose moiety (Fahey et al. 2001). Glucosinolate variations are based on differentiation of the side chains. The enzyme involved in the hydrolysis of glucosinolate to form D-glucose, sulfate anion, and various bioactive compounds is myrosinase (Fenwick et al. 1982). When cells containing GLN are ruptured, GLN is hydrolyzed by myrosinase into glucosinolate breakdown products (GBPs) (VanEtten and Tookey 1978). The GBPs are 3-methoxybenzyl isothiocyanate (isothiocyanate), 3-methoxyphenylacetonitrile (nitrile), 2-(3-methoxyphenyl)ethanethioamide (thioamide), and 2-(3-methoxyphenyl) acetamide (acetamide) (Stevens et al. 2009). Isothiocyanate and nitrile, prominent glucosinolate degradation compounds in Brassicaceae and non-Brassicaceae plant families, have been reported to have herbicidal activity. The application of Brassicaceae seed meals inhibited seed germination of numerous species. For example, redroot pigweed (*Amaranthus retroflexus* L.), common chickweed (*Stellaria media* L. (Vill.)), and common lambsquarters (*Chenopodium album* L.) germination was inhibited by brown mustard (*Brassica juncea* L.) seed meal (Rice et al. 2007), and carrot (*Daucus carota* L.) germination was inhibited by yellow mustard (*Sinapis alba* L.) seed meal (Hansson et al. 2008). For MSM, the reports of its potential for use as a bioherbicide were on the weed species, downy brome (*Bromus tectorum* L.) (Machado 2007; Stevens et al. 2009), and velvetleaf (*Abutilon theophrasti* Medik.) (Vaughn et al. 1996).

This study focused on the potential use of MSM as a pre-emergent herbicide. The phytotoxic activity from glucosinolate degradation products depends on various factors including soil pH, soil temperature, glucosinolate concentration in seed meal, and the presence of myrosinase enzyme. Myrosinase is present in leaf, stem, root, and seed of

glucosinolate-containing plants and is separated from vacuoles containing glucosinolate substrate (Borek et al. 1996; Fenwick et al. 1982; Thangstad et al. 1991). It is also present in soil microorganisms (Gimsing and Kirkegaard 2009; Rakariyatham et al. 2005; Sakorn et al. 2002). During the oil extraction process, myrosinase can be denatured by heat. The addition of seeds to MSM can result in the quantitative conversion of GLN to GBPs (Stevens et al. 2009). To preserve the bioactivity of glucosinolates, the appropriate way to apply a glucosinolate-containing seedmeal is soil incorporation (Gimsing and Kirkegaard 2009; Mathiessen and Kirkegaard 2006).

To our knowledge no investigation has been conducted on the temporal change in biodegradation of GLN and GBPs under soil conditions or on the identification of other possible GLN-related compounds. In this study, we compared the effect of MSM to other Brassicaceae seed meals on seed germination and growth suppression, evaluated the phytotoxicity of soil amended with either non-activated or activated MSM on seed emergence and growth, determined fate and persistence of GLN and GBPs in soil, and determined the effect of each GBP on seed emergence and growth.

# MATERIALS AND METHODS

## Materials

Seed meals of brown mustard[1] (Brassicaceae: *B. juncea* (L.) Czern.), camelina[2] (Brassicaceae: *Camelina sativa* (L.) Crantz), and meadowfoam[3] (Limnanthaceae: *L. alba*) were used in these studies. Seed meals were processed using a coffee grinder[4] and sieved through 1-mm mesh before use.

Soil was collected at 0-20 cm depth in 2010 from a site near Sweet Home, Oregon, USA (44º 25´ 5˝ N, 122º 42´ 43˝ W), where no herbicide application had been made for at least 4 years. The soil was a Newburg sandy loam (coarse-loamy, mesic Fluventic Haploxerolls) with an organic matter content of 3.2% and pH of 6.1. The soil was ground, passed through a 2-mm sieve, air-dried for 7 d, and kept in a closed container until use.

## Petri Dish Bioassay

Growth chamber studies were conducted using a completely randomized design with four replications. Twenty g of clean sand[5] (50 mesh particle size) were placed into 9-cm diameter Petri dishes. Brown mustard, camelina, non-activated meadowfoam, and activated meadowfoam seed meals were tested. A gram of ground seed meal was added on top of the sand in each dish and then mixed thoroughly. Activated MSM was produced by adding 1% by weight of freshly ground meadowfoam seed into MSM in order to provide active myrosinase (Stevens et al. 2009). Seed meals, brown mustard, camelina,

and non-activated meadowfoam contained only ground meal. Unamended sand was used as a control.

Because there are differences in water absorption for each seed meal, the water absorption for each was measured by modification of an American Association of Cereal Chemists (AACC) method for solvent retention capacity of flours (AACC International 2009). Briefly, 3 g of ground seed meal was put into a 50-ml centrifuge tube and 21 ml deionized water was added. The tube was capped, shaken vigorously to suspend meal, and allowed to solvate and swell for 20 min by shaking every 5 min. The mixture was centrifuged at 1000 x *g* for 15 min and the supernatant discarded. The tube was drained at a 90º angle on filter paper for 10 min before weighing. The water absorption was determined by subtracting the mass of meal before and after adding water. The average water absorption per gram of seed meal was 3.3±0.01, 3.7±0.03, and 8.0±0.09 ml for meadowfoam, brown mustard, and camelina, respectively.

For the control, 8 ml of deionized water was added to a Petri dish. Based on the water absorption capacity for each seed meal, the amount of deionized water per Petri dish was 11.3, 11.3, 11.7, and 16 ml for meadowfoam, activated meadowfoam, brown mustard, and camelina seed meal, respectively. A Whatman No.1 filter paper was placed on top of sand. Leaf lettuce[6] (*Lactuca sativa* L. 'Black Seeded Simpson') was used as an indicator species for all experiments in this study because of its rapid germination and its sensitivity to allelochemicals (Macías et al. 2000). Twenty-five lettuce seeds (93% germination) were placed equally spaced on the filter paper in each Petri dish. The Petri dishes were placed in the incubator with 20/15 ºC day/night temperature and a 14 hr photoperiod. Seeds were counted as germinated when the hypocotyl, radicle, or

hypocotyl plus radicle measured 2 mm and seedling growth was evaluated by measuring radicle and hypocotyl length on day 7. The experiment was performed in a completely randomized design with four replications of each seed meal treatment. The experiment was repeated.

**Phytotoxicity of Meadowfoam Seed Meal**

Meadowfoam seed meal[3] was used at 3% by weight. Activated MSM was prepared as previously described. A filter paper was placed at the bottom of 132-ml pot to prevent soil loss. Pots were filled with 116.4 g of soil and amended with either 3.6 g of MSM or activated MSM. The untreated control was 120 g of soil without amendment. Lettuce seeds were sown nine seeds per pot at an approximate depth of 0.4 cm on 0, 6, and 12 days after MSM incorporation (DAI). Each pot was watered daily. Soil moisture in each pot was maintained at 37.5% by weighing the pots. The experiments were conducted in the greenhouse with 25/20 ºC day/night temperature and a 14 hr photoperiod. Seedling emergence was determined by counting plants daily for 14 days after planting (DAP). Shoot biomass was harvested 21 DAP, dried at 60 ºC for 72 hr, and weighed. The experiment was structured in a randomized complete block design with three replications. The experiment was repeated.

**Glucolimnanthin and Its Breakdown Products**

Soil incubation was conducted in a 15 ml centrifuge tube using a completely randomized design with three replications. Dry soil (1.94 g) was incubated with either 0.06 g of MSM or activated MSM in a tube. Tubes were laid horizontally after adding

750 µl of deionized water to thoroughly hydrate the seed meal amended soil. Non-amended soil was used as a control. The extraction method for extracting glucosinolate and its hydrolysis compounds from the soil was developed by modifying the method of Stevens et al. (2009). Glucolimnanthin (CAS 111810-95-8, S-(*β*-D-glucopyranosyl)-(*Z*)-2-(3-methoxyphenyl)-*N*-(sulfooxy)ethanimidothioic acid potassium salt) was extracted from meadowfoam seed meal. 3-Methoxybenzyl isothiocyanate[7] (isothiocyanate), 3-methoxyphenylacetonitrile[8] (nitrile), and 3-methoxyphenylacetic acid[9] were used as standards for GLN and GBP analyses.

Each incubated soil tube received 6 ml of 70% methanol. The tube was shaken, sonicated for 10 min, and allowed to stand for 60 min. The mixture was centrifuged for 5 min at 3,000 rpm. The supernatant was centrifuged for 10 min at 13,000 rpm. The methanol concentration in the supernatant was increased to 90% to prevent further enzymatic degradation of GLN. The analyses of GLN and GBPs were performed using high performance liquid chromatography (HPLC) as described by Stevens et al. (2009). The injection volume was 30 µL. A Waters 2996 photodiode array detector[10] (210 to 500 nm) at 274 nm was used to calculate peak areas for all compounds. Analyte concentrations were determined from calibration curves constructed for each analyte using the external standard method as described by Stevens et al. (2009). The GLN and GBPs were quantified on 0, 1, 2, 3, 6, 12, and 18 DAI. The experiment was performed in a completely randomized design with three replications. The experiment was repeated.

**Isolation and Identification of an Unknown Metabolite**

An unknown metabolite was detected in soil amended with MSM and activated MSM on 2 and 3 DAI, respectively, during HPLC analyses of GLN, nitrile, and isothiocyanate. Isolation and identification of the unknown metabolite were performed using the following procedures. MSM at 3% by weight was added to a dry soil, mixed thoroughly, and 30% water added to a 11 x 11 x 3.5 cm plastic box. The MSM amended soil was incubated in the sealed germination box at room temperature for 4 d before extraction. The moist soil was transferred into a glass centrifuge tube and dichloromethane was added in a ratio of 1.5 ml to 1 g moist soil. The tube was shaken, sonicated for 3 min, and allowed to stand for 15 min. The supernatant was transferred to a glass tube and the extraction was repeated for 2 more cycles. The supernatants were combined and then dried using a rotary evaporator. The resultant suspension was redissolved in methanol at a ratio of 2 ml methanol to 1 g equivalent dry soil. The methanolic resuspension was extracted with hexane 3 times. The suspension in methanol was collected, evaporated using a rotary evaporator, and resuspended in methanol before purification using a Sephadex LH-20 column[12] eluted with 100% degas methanol at a flow rate of 1.6 ml $min^{-1}$. Elution was monitored using HPLC as previously described (Stevens et al. 2009).

The unknown metabolite fractions were collected and concentrated using a rotary evaporator. The suspension containing the unknown metabolite was redissolved in acetonitrile and separated on a 250 x 4.6 mm LiChrospher 5 μm C18 column[11] eluted with a linear gradient of 35 to 40% of 0.1% formic acid in acetonitrile and aqueous 0.1% formic acid in water at 1 ml $min^{-1}$. The eluted unknown was identified using LC-UV at

274 nm (SPD-10Avp UV-VIS Detector [13]) and LC-MS/MS (Triple TOF 5600[14]). The full-scan negative ion mode was performed at elution time of the unknown metabolite and followed by product ion scan of fragmentation components of *m/z* 165. The ionization was a DuoSpray source operated at 550 ºC and -4.5 kV using a declustering potential of -80 v and a collision energy of -20 eV. The commercial standard was analyzed using LC-UV and LC-MS/MS under the same conditions as the suspension extract. The retention times and mass spectra of the standard and the unknown metabolite were compared.

Nitrile was suspected to be the parent compound of the unknown metabolite. Therefore, the identification of the parent compound was determined by preparing a 20 g soil in a 9-cm diameter Petri dish. Nitrile solution was added to the dish at a rate of 0.1 mg $g^{-1}$ soil with 30% soil moisture. The extraction method for extracting glucosinolate hydrolysis compounds from the soil was performed as previously described and followed for 12 d. Unamended soil was used as a control. The metabolite from soil amended with nitrile was analyzed using HPLC, LC-UV, and high resolution LC-MS/MS with negative product ion scan of fragmentation component of *m/z* 165 as previously described. The retention times and mass spectra of the commercial standard and the metabolite from soil amended with nitrile were compared.

**Germination Bioassay**

In a comparative assay of relative toxicity, each GBP was used to test the response of lettuce emergence and growth. A 9-cm diameter Petri dish served as a bioassay study chamber. Test compounds were dissolved in ethanol and diluted in

various concentrations. Test solutions (480 µl) were added on top of a 8.26-cm diameter germination blotter paper. Based on preliminary test, the following concentrations were chosen for dose-response study of the test compounds. Isothiocyanate[7] solution was prepared to deliver a concentration of 0, 0.048, 0.48, 0.96, 1.92, 2.88, 3.84, and 4.8 µmol plate$^{-1}$. Nitrile[8] solution was prepared to give a concentration of 0, 0.48, 4.8, 9.6, 19.2, 28.8, 38.4, 48, and 96 µmol plate$^{-1}$ and MPAA[9] solution was prepared at 0, 0.048, 0.48, 4.8, 9.6, 19.2, 28.8, 38.4, 48 µmol plate$^{-1}$. Control dishes received ethanol only. The ethanol added to each dish was allowed to evaporate for 3 hr in a hood, and then 6 ml of deionized water was added to the dish. Sixteen lettuce seeds were placed in a 4 x 4 grid on top of a germination blotter paper. The Petri dish was sealed with a layer of Parafilm. Lettuce seeds were grown in the incubator at 20/15 ºC day/night temperature with a 14 hr photoperiod. Seeds were counted as germinated when the hypocotyl, radicle, or hypocotyl plus radicle measured 2 mm and seedling growth was evaluated by measuring radicle and hypocotyl length on day 7. The experiment was performed in a completely randomized design with three replications of each test compound. The experiment was repeated.

**Statistical Analyses**

Lettuce germination was calculated as percent of sown seeds. The percentage of root and hypocotyl length reduction was calculated by dividing the average length of emerged seedlings in each treatment by the average length of emerged seedlings in the control treatment. Lettuce emergence and biomass and concentrations of GLN and GBPs

were analyzed using one-way analysis of variance (ANOVA) with means separated using a least significant difference (LSD) test at a 0.05 level PROC GLM in SAS v. 9.2[15].

For phytotoxicity of glucolimnanthin breakdown products on lettuce germination study, dose-response curves were obtained by a nonlinear regression using a log-logistic equation (Equation 1) (Seefeldt et al. 1995; Streibig et al. 1993),

$$y = C + (D - C)/\left[1 + (x/\mathrm{I}_{50})^{b}\right] \qquad [1]$$

where $y$ represents germination (percentage of untreated control) at GBP concentration $x$, $C$ is the mean response at the greatest GBP concentration (lower limit), $D$ is the mean response when the GBP concentration is zero (upper limit), $b$ is the slope of the line at $I_{50}$, and $I_{50}$ is the GBP concentration required for 50% germination reduction.

The regression parameters, 95% confidence interval for each GBP, and lack of fit test were obtained using the package drc (Ritz and Streibig 2005, 2012) in the statistical program R v. 3.0.1 (R Core Team 2013). The relative $I_{50}$ level was calculated by the ratio of the $I_{50}$ of one GBP to another GBP.

# RESULTS

## Petri Dish Bioassay

In the growth chamber study, lettuce emergence and growth differed among seed meal types (Table 2.1). Lettuce emergence and growth were reduced by all amendment materials compared to the unamended control. Radicle length was more inhibited than hypocotyl length. Brown mustard and activated MSM completely inhibited lettuce emergence. Non-activated MSM and camelina meal inhibited radicle and hypocotyl length 90 and 59%, respectively, compared to the unamended control.

## Phytotoxicity of Meadowfoam Seed Meal

Lettuce emergence and growth were different among amendment materials and planting times (Table 2.2). For the 0 DAI planting date, lettuce emergence was different between MSM and activated MSM ($p < 0.05$). Lettuce emergence reduction was 55% for activated MSM compared to the untreated control. Some lettuce seedlings emerged but their growth was inhibited in MSM treatments. Emergence rate of the untreated control on 0 DAI planting date was less than on the 6 and 12 DAI planting dates. However, the biomass was not affected. On the 0 DAI, the average biomass of each lettuce seedling in the untreated control was 57% and 72% greater than those in MSM and activated MSM treatments, respectively. No differences in lettuce emergence and biomass were found between MSM forms at the 6 DAI planting date ($p > 0.05$). On the 12 DAI planting date, there was no difference in lettuce emergence but there was a difference in lettuce biomass. Total and average size of an individual lettuce seedling on the 12 DAI planting

date was greater in MSM compared to untreated control and activated MSM. On 0 DAI planting date, emerged lettuce seedlings were observed 2 DAP in unamended soil, while no lettuce seedling emerged until 6 DAP in activated MSM amended soil (Figure 2.1). At 14 DAP, there was 55% lettuce emergence inhibition in the activated MSM treatment compared to the untreated control.

**Glucolimnanthin and Its Breakdown Products**

Based on quantification of glucosinolate and its hydrolysis products (Figure 2.2), the glucosinolate was metabolized within 24 hr for activated MSM and 6 d for MSM treatments (Figure 2.3). No detection of GLN or GBP was found in non-amended soil. Nitrile concentrations were not significantly changed within 3 DAI in either MSM or activated MSM ($p > 0.05$). Nitrile concentrations were undetectable at 18 d for MSM and 12 d for activated MSM treatments. Isothiocyanate production was not detected in non-activated MSM (Figure 2.3A). Isothiocyanate concentrations in the activated MSM occurred within 0.5 hr, were greatest at 24 hr, dropped to less than half of the maximum at 48 hr, and were undetectable at 72 hr (Figure 2.3B). 3-Methoxyphenylacetic acid (MPAA), a previously unknown metabolite of GLN appeared on 2 DAI of MSM and 3 DAI of activated MSM. While GLN and other GBPs decreased on 3 DAI, MPAA increased. MPAA occurred last and remained detectable until 18 DAI. Maximum MPAA concentration was on 6 DAI at 0.38±0.057 μmol $g^{-1}$ soil for MSM and on 12 DAI at 0.24±0.058 μmol $g^{-1}$ soil for activated MSM. Its identity was confirmed by LC-UV and high resolution LC-MS/MS with an error 2.4 ppm compared to the exact mass of MPAA (Figure 2.4A). MPAA was derived from the nitrile based on the results of soil incubation

with nitrile with an error of 4.8 ppm when compared to the exact mass and product ion of the standard MPAA (Figure 2.4B). The observed spectrum of an MPAA standard had 2.4 ppm error compared to the calculated mass of MPAA (Figure 2.4C).

**Germination Bioassay**

A dose-response analysis on germination data was performed (Figure 2.5). The non-linear regression model of dose-response analysis on germination data was appropriate in compared to ANOVA (lack of fit test, $p = 0.50$). Isothiocyanate had the most effective herbicidal activity followed by MPAA and nitrile. The concentrations at 50% reduction of germination ($I_{50}$ ) and the 95% confidence intervals were 2.05 μmol plate$^{-1}$ (1.95-2.15 μmol plate$^{-1}$) for isothiocyanate, 11.90 μmol plate$^{-1}$ (10.91-12.89 μmol plate$^{-1}$) for MPAA, and 25.73 μmol plate$^{-1}$ (24.46-27.00 μmol plate$^{-1}$) for nitrile. The relative potency $I_{50}$ level of isothiocyanate was 5.8±0.25 and 12.5±0.40 times greater than MPAA and nitrile, respectively. The relative activity at $I_{50}$ level of MPAA was 2.2±0.09 times greater than nitrile ($p < 0.05$). Both hypocotyl and radicle length decreased with increasing GBP concentrations (Figure 2.6). All GBPs had more inhibitory effects on radicle length than hypocotyl length ($p = 0.04$).

## DISCUSSION

Brown mustard and activated MSM had the most potent herbicide effects providing greater suppression of lettuce emergence and growth. Although the lettuce germination was not completely inhibited in non-activated MSM and camelina seed meal, lettuce injury was observed on root tips and subsequent plant growth was suppressed. The difference between effects on emergence and overall growth suppression was previously reported with glucosinolate-containing seed meal (Brown and Morra 2005). The various effects of seed meals depend on seed meal species, application rates, target species, and growth characteristics of target species (Meyer et al. 2011). In this study, we focused on the differential effect among seed meals from different species. Glucosinolate breakdown products have been reported for their herbicidal effects (Brown and Morra 2005; Fahey et al. 2001; Vaughn 1999). However, glucosinolate structure and its degradation depended mainly on plant species variation (Fahey et al. 2001). Brown and Morra (1996) suggested that the seed germination inhibition was not solely due to glucosinolate concentration but also glucosinolate structure. Higher concentrations of glucosinolates and their degradation compounds were associated with more potent herbicide effects (Morra and Kirkegaard 2002). Reported glucosinolate levels in brown mustard were as high as 126.1 μmol $g^{-1}$ of meal (Rice et al. 2007) followed by the glucosinolate glucolimnanthin from meadowfoam seed meal at 75.2 μmol $g^{-1}$ of meal (Stevens et al. 2009). Camelina meal has naturally low glucosinolate resulting in less phytotoxic compounds which make it appropriate for animal feed (Erhensing et al. 2008; Kakani et al. 2012).

Greenhouse studies were conducted to evaluate the direct use of MSM as a soil amendment material for inhibition of seed emergence and growth. Activated MSM applied as a soil amendment had greater phytotoxicity on lettuce emergence and growth than non-activated MSM when lettuce seeds were sown on 0 DAI. Direct application of MSM as a soil amendment inhibited lettuce growth by 60% but had a minor effect on emergence. It is possible that myrosinase from soil microorganisms was insufficient to cause a lethal dose on lettuce. However, adding myrosinase containing freshly ground meadowfoam seed increased phytotoxicity and suppressed growth by up to 90% and emergence by 55% compared to the unamended treatment. There was a fertilizer effect from MSM addition on planting dates of 6 and 12 DAI, especially in non-activated MSM. Besides GLN contents, MSM provides sources of 25% protein and 22% fiber (Purdy and Craig 1987) which are utilized as nitrogen and carbon sources. The low number of emerged lettuce seedlings in all treatments on planting date 0 DAI was possibly due to buried seeds during the initial irrigation. When planted 6 and 12 d later, more settled soil may have resulted in increased emergence.

After 6 d in the soil, the soil microorganisms may have decomposed organic material from the MSM into plant-available nutrient forms. The lettuce growth on planting date 12 DAI was greater than on 6 DAI due to increased nutrient availability. Similar fertilizer affects were found after other Brassicaceae seed meal applications (Johnson-Maynard et al. 2005; Rice et al. 2007). The lettuce growth was greater in non-activated MSM than in activated MSM. The pathway of producing phytotoxic compounds in activated MSM possibly slowed the process of organic degradation by inhibiting the growth of soil microorganisms.

The GLN and GBP concentrations from MSM or activated MSM were observed over 18 d of soil incorporation. Isothiocyanate production occurred within 30 min after 37.5% moisture was added to soil amended with activated MSM. GLN was hydrolyzed to isothiocyanate by mediation of active myrosinase from freshly ground meadowfoam seed (Stevens et al. 2009). Maximal concentrations of isothiocyanate were observed at 24 hr, after which they decreased by more than 90% of the maximum by 48 hr. The degradation of isothiocyanate in soil incorporated with activated MSM happened quickly compared to soil incubation with glucosinolate-containing tissues in other Brassicaceae. Morra and Kirkegaard (2002) reported isothiocynate concentrations extracted from soil treated with shoot and root tissues of rapeseed (*B. napus*) and brown mustard peaked at 24 hr and dropped more than 50% by 72 hr. Gardiner et al. (1999) extracted isothiocynate from field soil amended with rapeseed tissues and reported that isothiocyanate concentrations peaked at 30 hr after incorporation and then steeply decreased but lasted until 20 d. The fast degradation of isothiocyanate in MSM may make MSM less effective in terms of bioherbicide use compared to rapeseed tissues. Morra and Kirkegaard (2002) suggested that the detection of glucosinolate and its degradation compound varied over different soil chemical and physical characteristics, temperature, and moisture. GLN in non-activated MSM degraded within 6 d and isothiocyanate was not detected. It is possible that soil microorganisms in this soil did not produce sufficient myrosinase for GLN hydrolysis. Myrosinase activity in soil is probably from soil microorganisms (Gimsing and Kirkegaard 2009) and soil fungi in the genus *Aspergillus* have been shown to produce myrosinase (Rakariyatham et al. 2005; Sakorn et al. 2002). Morra and Kirkegaard (2002) suggested conditions to increase isothiocyanate production and its

retention in soil by increasing the disruption of tissue-containing glucosinolate and providing adequate moisture for the hydrolysis process. Isothiocyanate disappeared rapidly because it easily binds with free amino acids and proteins to form thiourea derivatives (Vaughn 1999).

Nitrile is a thermally-induced degradation product of GLN present in MSM. Nitrile concentrations decreased from 3 to 18 d after incorporation in non-activated MSM and from 3 to 12 d after incorporation in activated MSM. From 30 min to 3 d after incorporation, nitrile concentrations with MSM or activated MSM were not different. Gardiner et al. (1999) reported that nitrile was metabolized from glucosinolate degradation in rapeseed tissues and had a pattern of degradation compounds similar to isothiocyanate but in lesser amounts. Nitrile concentrations peaked at 30 hr after incorporation and then declined until there was no detection over 20 d. Stevens et al. (2009) suggested that nitrile production in MSM can be induced by heat and steam during oil extraction, but is only minimally produced by enzymatic degradation of GLN. They measured nitrile concentrations of 23 μmol $g^{-1}$ in MSM. In our study, nitrile was detected within 30 min at concentrations of 0.5 μmol $g^{-1}$ soil in both MSM and activated MSM.

After GLN and other GBPs decreased, MPAA was detected at 48 hr for MSM and 72 hr for activated MSM. MPAA concentrations were lower compared to other GBPs. Time to maximum MPAA concentration varied across treatments. MPAA concentration in MSM and activated MSM peaked when nitrile decreased by 27% and 100%, respectively. On 18 d after incorporation, MPAA concentrations in MSM and activated MSM were near the limit of detection. There was no MPAA detection in either MSM or activated MSM without soil being present.

The metabolite from soil amended with nitrile was determined to confirm whether nitrile was the parent compound of MPAA. The metabolite was compared to the exact mass of standard MPAA. The acceptable range of mass errors in high-resolution mass spectrometry is within 10 ppm and in our study, the error was 4.8 ppm (Russell and Edmondson 1997). Nitrilase from soil microorganisms catalyzes nitrile hydrolysis to carboxylic acids (O'Reilly and Turner 2003). However, the conversion rate of nitrile to MPAA was low probably due to poor solubility of nitrile in water (Brady et al. 2004). Nitrile can be metabolized to either amide or carboxylic acid (O'Reilly and Turner 2003). However, there was no detection of the amide compound in our study. The cause of the preferred metabolism pathway is unknown.

Potential herbicical efficacy of each GBP was of interest. The required concentration of GBPs at a given dose for suppression of seed emergence and growth is important for developing MSM as a bioherbicide. Commercially available GBPs were used as a reference point for the evaluation of their phytotoxicity (Morra and Kirkegaard 2002). All GBPs (nitrile, MPAA, and isothiocyanate) showed potent inhibition of lettuce germination and growth but $I_{50}$ concentrations varied. Isothiocyanate had the most potent bioherbicidal activity but its presence was very short-lived, as previously described. Nitrile and MPAA remained longer but their $I_{50}$ concentrations were less than isothiocyanate. Others have confirmed that isothiocyanate has the most potent phytotoxicity for pest control (Bartelt and Mikolajczak 1989; Brown and Morra 1996; Morra and Kirkegaard 2002; Vaughn et al. 2006; Zasada et al. 2012). Isothiocyanate is a general biocide that can interact with protein or amino acid to form stable products (Brown and Morra 1996). Vaughn et al. (1996) reported that although much higher

toxicity of isothiocyanate was observed, the large amount of nitrile in MSM seed meal was possibly a driving factor in the radicle inhibition of velvetleaf and wheat. Stevens et al. (2009) determined that nitrile provided the highest inhibitory effects on coleoptile emergence of downy brome. The different levels of seed emergence and growth inhibition caused by isothiocyanate and nitrile vary by experimental conditions and target species. In our case, activated MSM suppressed lettuce germination and growth. It is possible that phytotoxicity does not depend on only a single chemical compound. A combination of all GBPs may result in the total observed phytotoxicity. However, the synergistic or antagonistic response of GBP combinations for herbicidal activity is unknown.

MSM has both bioherbicidal and fertilizer affects. Soil amended with 3% by weight of activated MSM provided the initial suppression of seed emergence and growth. The most potent activity lasted for 6 d after activated MSM application. The rapid degradation of bioactive compounds in the soil should allow the use of activated MSM for weed control preplant if there is an adequate delay before planting the crop to prevent crop injury. Short season and/or transplanted crops would be recommended because good stand establishment helps to protect the crop from remaining allelochemical and increases competitiveness to later emerging weeds. A repeat application of MSM may be needed to extend the length of MSM phytotoxicity for late-season weed control. Besides the optimal timing and activation method, the development of meadowfoam seed meal as a bioherbicide still requires more data on rate, application method, and evaluation under field conditions.

## SOURCES OF MATERIALS

[1] Brown mustard meal, Brassica Breeding and Research Group, University of Idaho, 875 Perimeter Dr. MS 2339, Moscow, ID 83844.

[2] Camelina meal, Willamette Biomass Processor, Inc., 1055 S Pacific Hwy W, Rickreall, OR 97371.

[3] Meadowfoam seed meal, Natural Plant Products, Inc., 707 13th St. SE, Suite 275, Salem, OR 97301.

[4] Coffee grinder, Proctor Silex, Hamilton Beach Brands, Inc., 261 Yadkin Rd., Southern Pines, NC 28387.

[5] Sand, Willamette Graystone, 121 SW McKenzie Ave., Corvallis, OR 97333.

[6] Leaf lettuce 'Black Seeded Simpson', Planation Products Inc., 202 S. Washington St., Norton, MA 02766.

[7] 3-Methoxybenzyl isothiocyanate, Oakwood Products, West Columbia, SC 29172

[8] 3-Methoxyphenylacetonitrile, Sigma-Aldrich Chemicals, St. Louis, MO 63178.

[9] 3-Methoxyphenylacetic acid, TCI America, Portland, OR 97203.

[10] Waters 2996 photodiode array detector, Waters Corporation, 34 Maple Street Milford, MA 01757.

[11] Sephadex LH-20, Sigma-Aldrich Chemicals, St. Louis, MO 63178.

[12] 250 x 4.6 mm LiChrospher 5 μm C18 column, Sigma-Aldrich Chemicals, St. Louis, MO 63178.

[13] SPD-10Avp UV-VIS Detector, Shimadzu Scientific Instruments, Inc., 7102 Riverwood Dr., Columbia, MD 21046.

[14] Triple TOF 5600, AB SCIEX, 500 Old Connecticut Path, Framingham, MA 01701.

[15] SAS version 9.2, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513.

## LITERATURE CITED

AACC International. 2009. Approved methods of the AACC (11th ed). St. Paul, MN: American Association of Cereal Chemists (Method 56-11.02).

Bartelt, R. J. and K. L. Mikolajczak. 1989. Toxicity of compounds derived from *Limnanthes alba* seed to fall armyworm (Lepidoptera: Noctuidae) and European corn borer (Lepidoptera: Pyralidae) Larvae. J. Econ. Entomol. 82: 1054-1060.

Borek, V., M. J. Morra, and J. P. McCaffrey. 1996. Myrosinase activity in soil extracts. Soil Sci. Soc. Am. J. 60: 1792-1797.

Brady, D., A. Beeton, J. Zeevaart, C. Kgaje, F. Rantwijk, and R. A. Sheldon. 2004. Characterisation of nitrilase and nitrile hydratase biocatalytic systems. Appl. Microbiol. Biotechnol. 64: 76-85.

Brown, P. D. and M. J. Morra. 1996. Hydrolysis products of glucosinolates in *Brassica napus* tissues as inhibitors of seed germination. Plant Soil 181: 307-316.

Brown, J. and M. J. Morra. 2005. Glucosinolate-containing seed meal as a soil amendment to control plant pests. Subcontract Report 2000-2002, Univ. of Idaho, Moscow, Idaho. 99 p.

Burg, D. A. and R. Kleiman. 1991. Preparation of meadowfoam dimer acids and dimer esters and their use as lubricants. J. Am. Oil Chem. Soc. 68:600-603.

Ehrensing, D. T. and S. O. Guy. 2008. Oil seed crops: camelina. Oregon State University Extension Service. EM 8953-E.

Ehrensing, D. T., G. D. Jolliff, J. M. Crane, and R. S. Karow. 1997. Growing meadowfoam in the Willamette Valley. Oregon State University Extension Service. EM 8567.

Fahey, J. W., A. T. Zalcmann, and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56: 5-51.

Fenwick, G. R., R. K. Heaney, and W. J. Mullin. 1982. Glucosinolates and their breakdown products in food and food plants. Crit. Rev. Food. Sci. 18: 123-201.

Gardiner, J. B., M. J. Morra, C. V. Eberlein, P. D. Brown, and V. Borek. 1999. Allelochemicals released in soil following incorporation of rapeseed (*Brassica napus*) green manures. J. Agric. Food Chem. 47: 3837-3842.

Gimsing, A. and J. Kirkegaard. 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochem. Rev. 8: 299-310.

Hansson, D., M. J. Morra, V. Borek, A. J. Snyder, J. L. Johnson-Maynard, and D. C. Thill. 2008. Ionic thiocyanate ($SCN^-$) production, fate, and phytotoxicity in soil amended with Brassicaceae seed meals. J. Agric. Food Chem. 56: 3912-3917.

Hirsinger, F. 1989. New annual oil crops. Pages 518-532 in G. Röbbelen, R. K. Downey, and A. Ashri, eds. Oil Crops of the World. New York: McGraw-Hill.

Johnson-Maynard, J. M., Morra, L. Dandurand, C. William, and M. J. Butters. 2005. Brassicaceae seed meal application for weed reduction and improved nitrogen management in organic farming systems. Project report, University of Idaho, Moscow, ID.

Kakani, R., J. Fowler, A.-U. Haq, E. Murphy, T. Rosenberger, M. Berhow, and C. A. Bailey. 2012. Camelina meal increases egg n-3 fatty acid content without altering quality or production in laying hens. Lipids 47: 519-526.

Kleiman, R. 1990. Chemistry of new industrial oilseed crops. Pages 198-199 in J. Janick and J. E. Simon, eds. Advances in New Crops. Portland, OR: Timber Press.

Knapp, S. J. and J. M. Crane. 1995. Fatty acid diversity of section Inflexae *Limnanthes* (meadowfoam). Ind. Crops Prod. 4: 219-227.

Linderman, R. G., E. A. Davis, and C. J. Masters. 2007. Response of conifer seedlings to potting medium amendment with meadowfoam seed meal. Pages 138-142 in J. Janick and A. Whipkey, eds. Issues in New Crops and New Uses. Alexandria, VA: ASHS Press.

Machado, S. 2007. Allelopathic potential of various plant species on downy brome: implications for weed control in wheat production. Agron. J. 99: 127-132.

Macías, F. A., D. Castellano, and J. M. G. Molinillo. 2000. Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. J. Agric. Food Chem. 48: 2512-2521.

Matthiessen, J. and J. Kirkegaard. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Crit. Rev. Plant Sci. 25: 235-265.

Meyer, S. L. F., I. A. Zasada, S. B. Orisajo, and M. J. Morra. 2011. Mustard seed meal mixtures: management of *Meloidogyne incognita* on pepper and potential phytotoxicity. J. Nematol. 43: 7-15.

Morra, M. J. and J. A. Kirkegaard. 2002. Isothiocyanate release from soil-incorporated *Brassica* tissues. Soil Biol. Biochem. 34: 1683-1690.

O'Reilly, C. and P. D. Turner. 2003. A review: the nitrilase family of CN hydrolyzing enzymes - a comparative study. J. Appl. Microbiol. 95: 1161-1174.

Purdy, R. H. and C. D. Craig. 1987. Meadowfoam: new source of long-chain fatty acids. J. Am. Oil Chem. Soc. 64: 1493-1498.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/.

Rakariyatham, N., B. Butrindr, H. Niamsup, and L. Shank. 2005. Screening of filamentous fungi for production of myrosinase. Brazilian J. Microbiol. 36: 242-245.

Rice, A. R., J. L. Johnson-Maynard, D. C. Thill, and M. J. Morra. 2007. Vegetable crop emergence and weed control following amendment with different Brassicaceae seed meals. Renew. Agric. Food Syst. 22: 204-212.

Ritz, C. and J. C. Streibig. 2005. Bioassay Analysis using R. J. Statist. Software. 12: 1-22.

Ritz, C. and J. C. Streibig. 2012. Dose response curves and other nonlinear curves in Weed Science and Ecotoxicology with the add-on package drc in R. Available at http://www.bioassay.dk/index-filer/start/DraftDrcManual.pdf. Accessed: May 26, 2013.

Russell, D. H. and R. D. Edmondson. 1997. High-resolution mass spectrometry and accurate mass measurements with emphasis on the characterization of peptides and proteins by matrix-assisted laser desorption/ionization time-of-fight mass spectrometry J. Mass Spec. 32: 263-276.

Sakorn, P., N. Rakariyatham, H. Niamsup, and P. Nongkunsarn. 2002. Rapid detection of myrosinase-producing fungi: a plate method based on opaque barium sulphate formation. World J. Microbiol. Biotech. 18: 73-74.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Tech. 9: 218-227.

Stevens, J. F., R. L., Reed, S. Alber, L. Pritchett, and S. Machado. 2009. Herbicidal activity of glucosinolate degradation products in fermented meadowfoam (*Limnanthes alba*) seed meal. J. Agric. Food Chem. 57: 1821-1826.

Steiner, J. J., G. W. Mueller-Warrant, S. M. Griffith, G. M. Banowetz, and G. W. Whittaker. 2006. Conservation practices in western Oregon perennial grass seed systems: II. meadowfoam rotation crop management. Agron. J. 98: 1501-1509.

Streibig, J. C., M. Rudemo, and J. E. Jensen. 1993. Dose-response curves and statistical models. Pages 29-53 in J. C. Streibig and P. Kudsk, eds. Herbicide Bioassay. Boca Raton, FL: CRC Press.

Thangstad, O. P., K. Evjen, and A. Bones. 1991. Immunogold-EM localization of myrosinase in Brassicaceae. Protoplama 161: 85-93.

VanEtten, C. H. and H. L. Tookey. 1978. Glucosinolates in cruciferous plants. Page 507-520 in R. F. Keeler, K. R. Van Kampen, and L. F. James, eds. Effects of Poisonous Plants on Livestock. New York: Academic Press.

Vaughn, S. F. 1999. Glucosinolates as natural pesticides. Pages 81-91 in H. G. Cutler and S. J. Cutler, eds. Biologically Active Natural Products: Agrochemicals. New York: CRC Press.

Vaughn, S. F., R. A. Boydston, and C. A. Mallory-Smith. 1996. Isolation and identification of (3-methoxyphenyl) acetonitrile as a phytotoxin from meadowfoam (*Limnanthes alba*) seed meal. J. Chem. Ecol. 22: 1939-1949.

Vaughn, S. F., D. E. Palmquist, S. M. Duval, and M. A. Berhow. 2006. Herbicidal activity of glucosinolate-containing seedmeals. Weed Sci. 54: 743-748.

Zasada, I. A., J. E. Weiland, R. L. Reed, and J. F. Stevens. 2012. Activity of meadowfoam (*Limnanthes alba*) seed meal glucolimnanthin degradation products against soilborne pathogens. J. Agric. Food Chem. 60: 339-345.

Table 2.1. Phytotoxicity effects of sand amended with different seed meals on lettuce germination and growth in Petri dish. Seed meal concentration was 4.8% by weight.

| Amendment materials | Lettuce emergence[a] | | Radicle | | | Hypocotyl | | |
|---|---|---|---|---|---|---|---|---|
| | | | Length | | Reduction[b] | Length | | Reduction |
| | % of sown seeds | | -----cm---- | | ----%---- | ----cm---- | | ----%---- |
| Unamended | 93(2.6) | a | 3.5(0.17) | a | - | 0.8(0.07) | a | - |
| Camelina | 81(4.0) | b | 0.3(0.01) | b | 91 | 0.3(0.06) | b | 59 |
| MSM[c] | 65(3.5) | c | 0.2(0.02) | b | 93 | 0.3(0.03) | b | 59 |
| Activated MSM[d] | 0 | d | 0 | c | 100 | 0 | c | 100 |
| Brown mustard | 0 | d | 0 | c | 100 | 0 | c | 100 |

[a] Data are represented as means with standard errors within parentheses. Different letters within a column indicate significant differences at the 0.05 level using Fisher's LSD.

[b] Percent reduction compared with unamended control.

[c] Meadowfoam seed meal.

[d] 1% freshly ground meadowfoam seed in total meadowfoam seed meal.

Table 2.2. Greenhouse studies on lettuce emergence and growth in soil amended with 3% by weight of non-activated or activated meadowfoam seed meal.

| Treatments | Lettuce emergence[a] | | | Lettuce biomass | | |
|---|---|---|---|---|---|---|
| | ---% of sown seeds--- | | | ---mg plant$^{-1}$--- | | |
| | Planted on 0 DAI[b] | | | | | |
| Unamended | 57.4 | (5.30) | a | 9.7 | (1.30) | a |
| MSM[c] | 50.0 | (10.24) | a | 4.1 | (0.87) | b |
| Activated MSM[d] | 25.9 | (4.68) | b | 2.7 | (1.00) | b |
| | Planted on 6 DAI | | | | | |
| Unamended | 79.6 | (5.30) | ns[e] | 9.4 | (0.89) | ns |
| MSM | 70.4 | (9.37) | | 13.9 | (3.29) | |
| Activated MSM | 77.8 | (2.87) | | 8.5 | (2.61) | |
| | Planted on 12 DAI | | | | | |
| Unamended | 81.5 | (8.45) | ns | 10.0 | (0.65) | b |
| MSM | 77.8 | (7.03) | | 19.3 | (2.44) | a |
| Activated MSM | 75.9 | (3.41) | | 10.9 | (1.83) | b |

[a] Data are represented as means with standard errors within parentheses. Different letters within a column indicate significant differences at the 0.05 level using Fisher's LSD within a planting date.

[b] Planting dates after meal incorporation (DAI).

[c] Meadowfoam seed meal

[d] 1% freshly ground meadowfoam seed to total meadowfoam seed meal.

[e] Not significant.

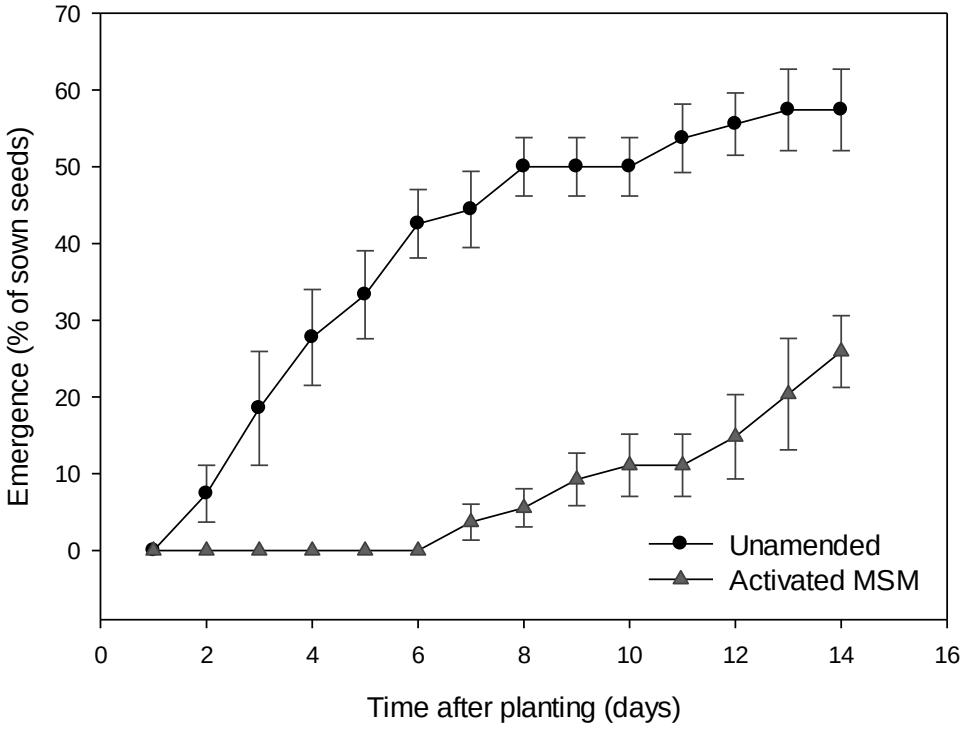



Figure 2.1. Lettuce emergence in percentage of sown seeds in unamended and activated meadowfoam amended soil. Lettuce seeds were sown after seed meal incorporation and emergence was observed for 14 days. Symbols and bars represent means and standard errors of sample between two studies ($n$ = 6).

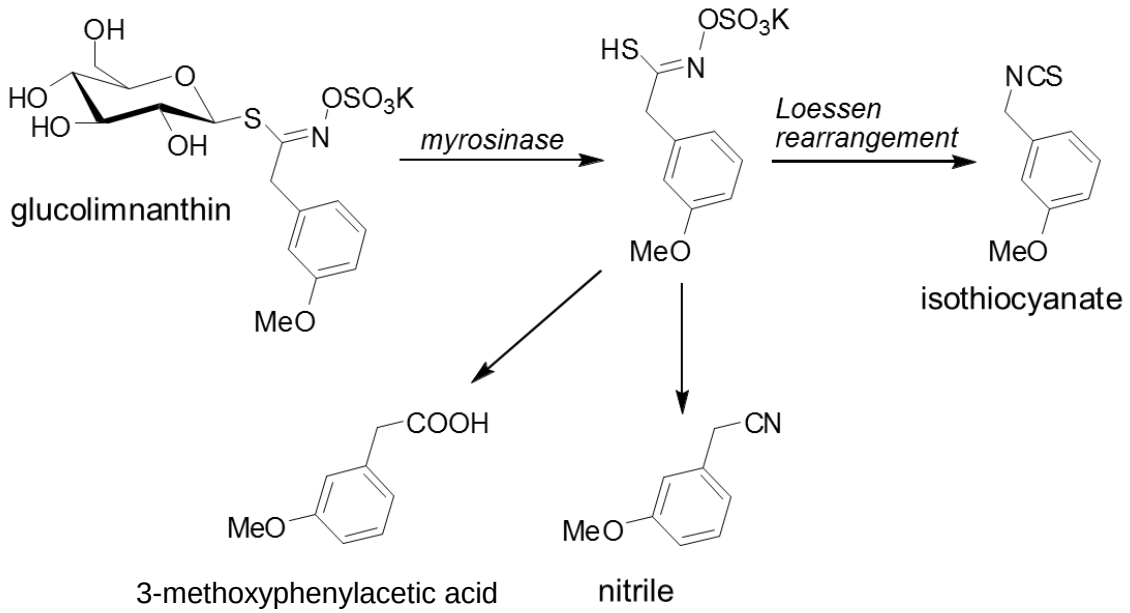



Figure 2.2. The production of glucolimnanthin breakdown products in soil amendment with meadowfoam seed meal.

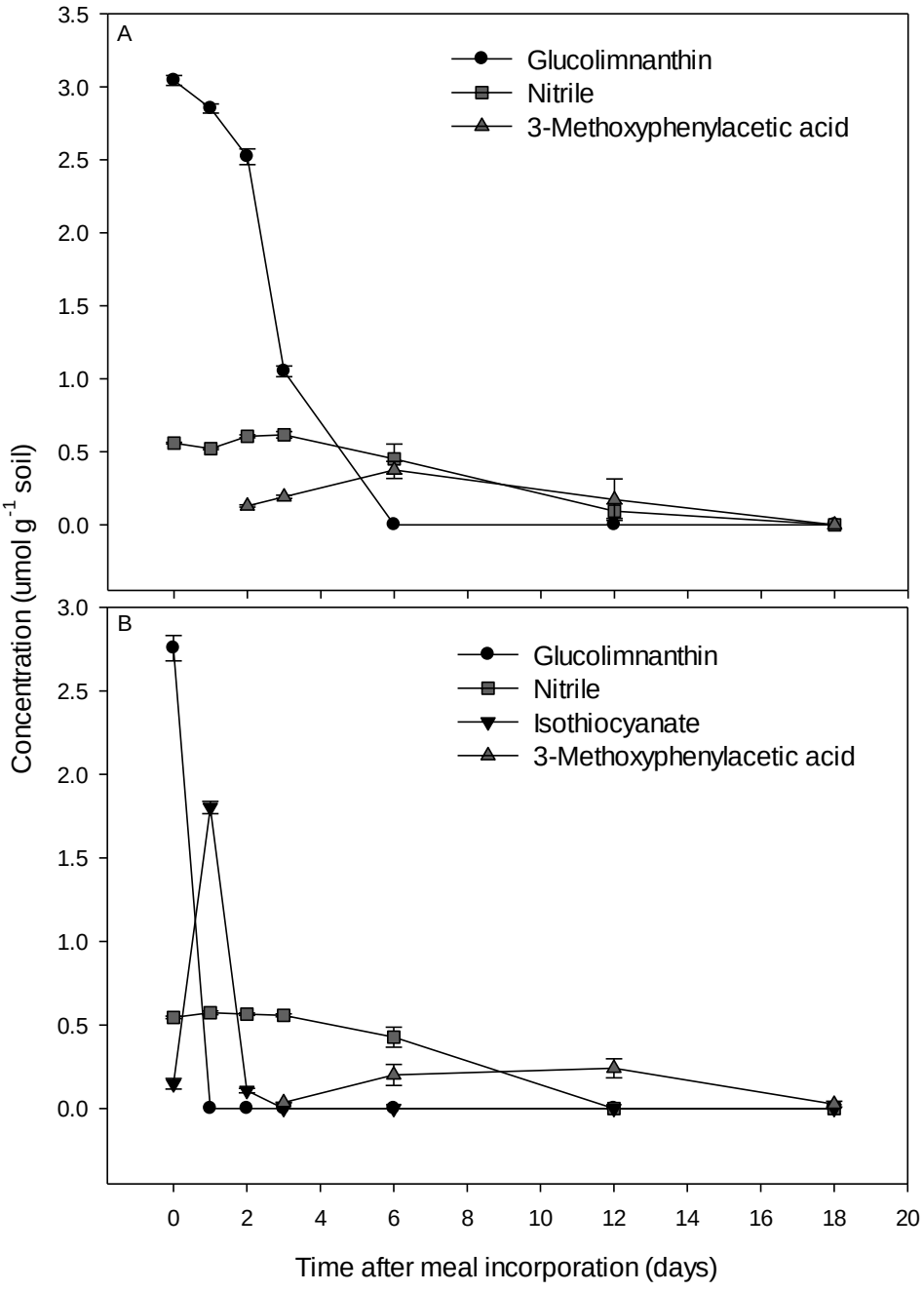



Figure 2.3. Concentrations of glucolimnanthin and its breakdown products in soil amended with meadowfoam seed meal (A) and activated meadowfoam seed meal (B). On day 0, the extraction started 30 minutes after meal incorporation. Symbols and bars represent means and standard errors of two studies ($n$ = 6).

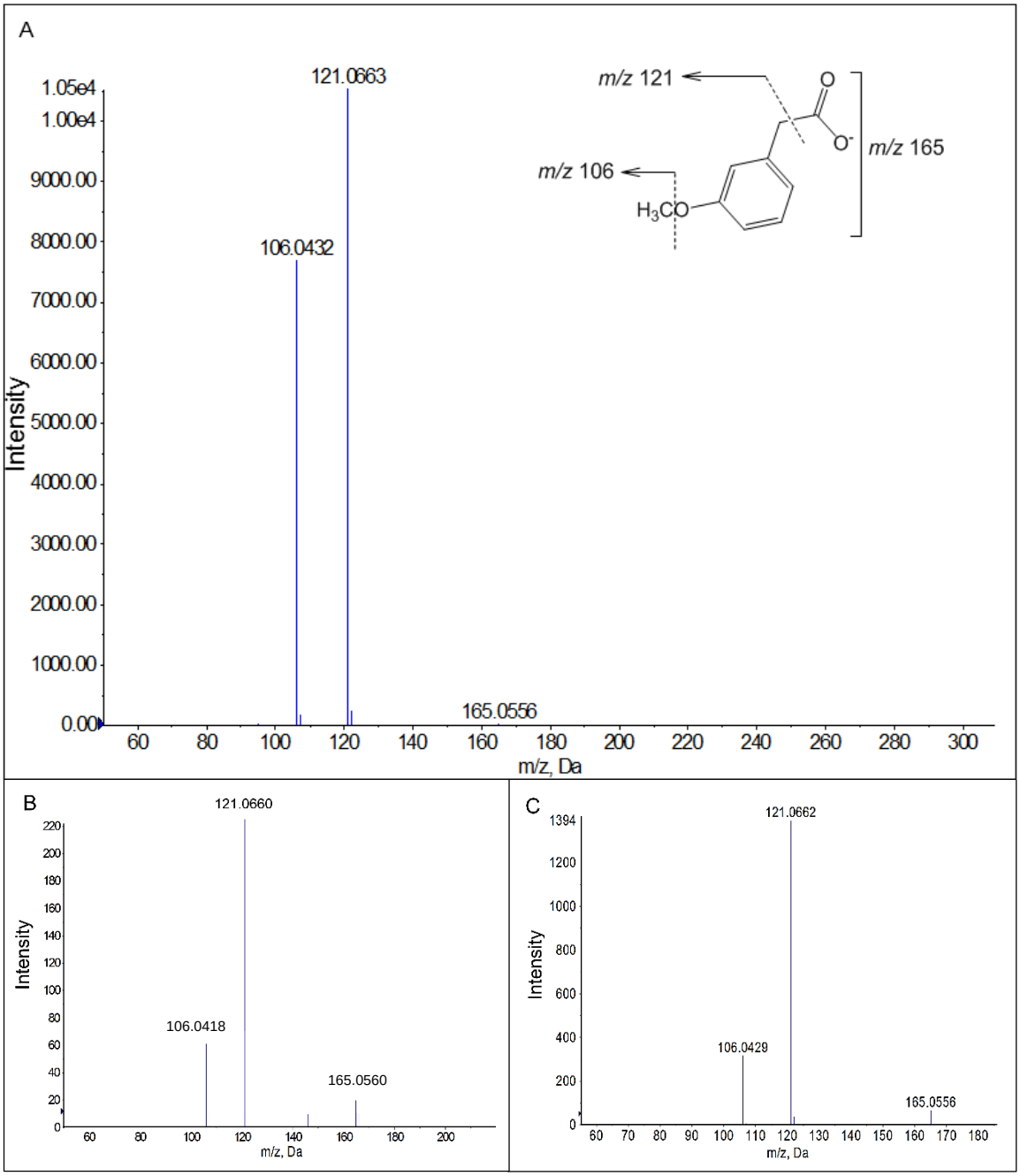



Figure 2.4. Mass spectrum of 3-methoxyphenylacetic acid (MPAA) identified using LC-MS/MS with negative product ion scan of *m/z* 165 using a Triple TOF 5600, calculated for $C_9H_9O_3$: 165.0552. Products were produced from soil amended with 3% by weight of meadowfoam seed meal (observed *m/z* 165.0556, 2.4 ppm error) (A); soil incubation with nitrile (observed *m/z* 165.0560, 4.8 ppm error) (B); and the spectrum of an MPAA standard (observed *m/z* 165.0556, 2.4 ppm error) (C).

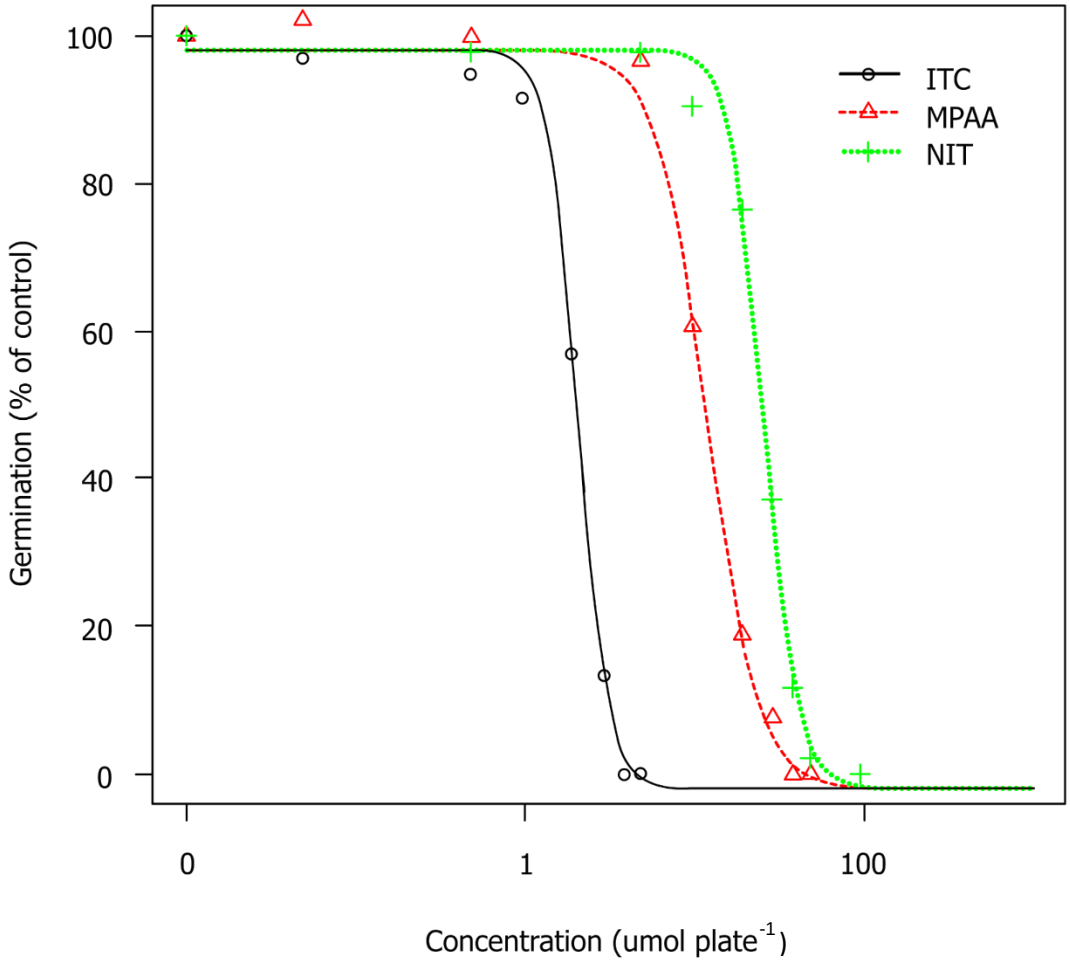



Figure 2.5. Phytotoxicity of glucolimnanthin breakdown products on lettuce germination in response to various concentrations. Symbols represent means of samples of two studies ($n$ = 6). ITC = isothiocyanate; MPAA = 3-methoxyphenylacetic acid; NIT = nitrile.

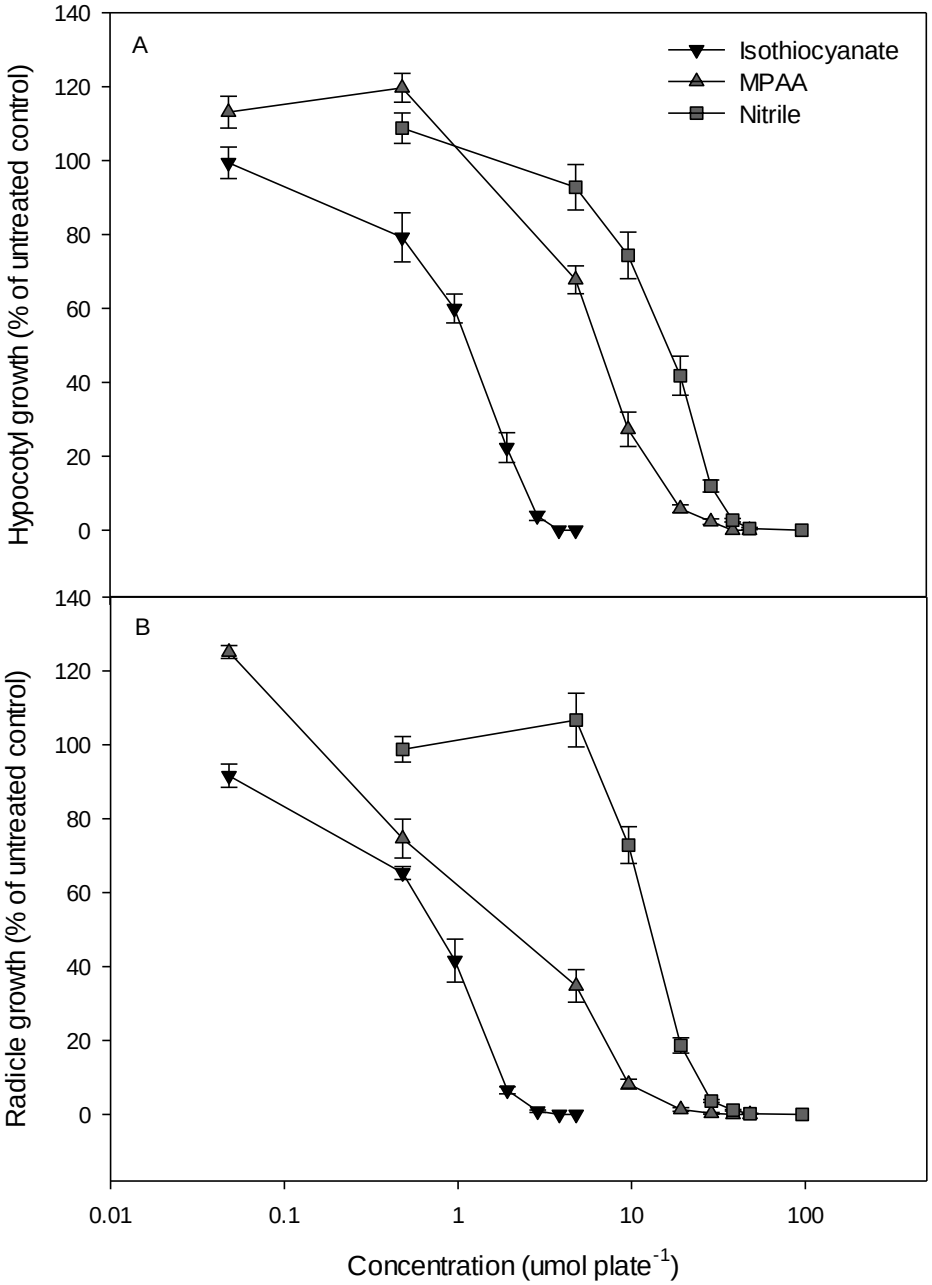



Figure 2.6. Phytotoxicity of glucolimnanthin breakdown products on lettuce hypocotyl length (A) and radicle length (B) in response to various concentrations. Symbols and bars represent means and standard errors of samples of two studies ($n$ = 6). MPAA = 3-methoxyphenylacetic acid.

# CHAPTER 3

## IMPACT OF DIFFERENT MEADOWFOAM SEED MEAL CONCENTRATIONS AND ACTIVATION FORM ON LETTUCE GROWTH, SOIL NUTRIENT, AND WEED COMMUNITY COMPOSITION

## ABSTRACT

In an organic farming system, fertilizer and weed control options are limited. Meadowfoam (*Limnanthes alba* Hartw. ex Benth) seed meal, a by-product after oil extraction, has potential uses for crop growth enhancement and weed control. The herbicidal effect of meadowfoam seed meal (MSM) is the result of a plant secondary metabolite, glucosinolate glucolimnanthin (GLN). Glucosinolate breakdown products (GBPs) have been reported to inhibit seed emergence and plant growth. Different concentrations (3, 5, and 7% by weight) and forms (non-activated and activated) of MSM were applied as soil amendments in studies using transplanted lettuce. No injury was observed on transplanted lettuce 7 days after MSM incorporation. Lettuce biomass and leaf nitrogen content were greater in seed meal amended treatments compared to the untreated control. Different MSM concentrations effected weed emergence, growth, and community composition, whereas no difference was observed between MSM forms. Weed emergence was inhibited at least 28% for 3% MSM and at least 54% for 5 and 7% MSM compared to the untreated control. Greater soil nitrate levels correlated with greater weed biomass in MSM-amended plots. Annual weeds were dominant in MSM-amended plots, whereas perennial weeds were found to a lesser degree in MSM-amended compared to untreated control plots. No herbicidal selectivity of MSM was detected for a particular weed species. Isothiocyanate, a potent herbicidal compound was found only in

5 and 7% activated MSM. Optimal timing and method of application of MSM should be further investigated to optimize both bioherbicide and fertilizer benefits.

# INTRODUCTION

Weed control options are limited in organic farming systems. Integration of cultural practices and mechanical methods can be an effective strategy for organic farmers. Cultural weed control methods include crop or variety selection, rotation, plant population and spacing, fertility, and irrigation (Monaco et al. 2002). Mechanical control methods include tilling, hand weeding, mowing, mulching, burning or flooding. Tillage is used to prepare a good soil bed and provides weed control. However, intensive tillage results in soil compaction, breakdown of soil structure, a shift in microbial activity and loss of organic matter (Martens and Martens 2002). Hand weeding is time consuming, costly, and labor intensive. Alternative options that can enhance crop growth and suppress weed pressure in organic farming are of public interest.

Meadowfoam (*Limnanthes alba* Hartw. ex Benth.) is an oil seed crop grown as a rotation seed crop in the Willamette Valley of Oregon (Ehrensing et al. 1997; Steiner et al. 2006). Meadowfoam is native to southern Oregon and northern California (Kleiman 1990). About 70% of the biomass of a seed remains following meadowfoam oil extraction. This by-product, known as meadowfoam seed meal (MSM), has limited commercial uses. MSM consists of 25% protein, 22% fiber, and 4% a plant secondary metabolite (Purdy and Craig 1987). The plant secondary metabolite in MSM is known as glucosinolate glucolimnanthin (GLN). The glucosinolate breakdown products (GBPs) occur when cells containing GLN are ruptured and GLN is hydrolyzed by myrosinase (VanEtten and Tookey 1978). The GBPs are 3-methoxybenzyl isothiocyanate (isothiocyanate), 3-methoxyphenylacetonitrile (nitrile), 2-(3-methoxyphenyl)

ethanethioamide (thioamide), and 2-(3-methoxyphenyl) acetamide (acetamide) (Stevens et al. 2009). Isothiocyanate and nitrile, have been reported to have herbicidal activity. MSM has some characteristics that suggest its potential utility in agriculture as a soil amendment to enhance plant growth (Linderman et al. 2007), suppress weeds (Machado 2007; Stevens et al. 2009; Vaughn et al. 1996; Vaughn et al. 2006), or inhibit soil pests such as nematodes (Zasada et al. 2012) and insects (Bartelt and Mikolajczak 1989).

The effectiveness of MSM as a soil amendment depends on its concentration. Low levels of MSM may stimulate growth of vegetable crops (Vaughn et al. 2008). Meadowfoam seed meal at concentrations of 1% to 2% by volume amended with a peat-based soil-less medium stimulated seedling growth of conifer species (Linderman et al. 2007). At MSM concentrations greater than 2% by volume or weight, inhibition of seed germination was observed (Linderman et al. 2007; Machado 2007; Stevens et al. 2009; Vaughn et al. 1996; Vaughn et al. 2006). Laboratory and greenhouse studies confirmed the herbicidal effect of MSM on seedling emergence and growth compared to a control (see Chapter 2; Machado 2007; Stevens et al. 2009; Vaughn et al. 1996). However, the best practices for use of the meal under field conditions as a bioherbicide still need to be identified. To preserve the bioactivity of glucosinolates, glucosinolate-containing seedmeal needs soil incorporation (Gimsing and Kirkegaard 2009; Mathiessen and Kirkegaard 2006). Adding 1% by weight of freshly ground meadowfoam seed to MSM provides active myrosinase and quantitative conversion of GLN to GBPs (Stevens et al. 2009).

The goal of this study was to investigate changes in crop growth, plant-available nitrogen, and weed species composition when varying concentrations of activated and

non-activated forms of MSM were applied to the soil. The specific objectives were to evaluate optimal timing after MSM application for crop safety, to determine whether the response of the weed community to MSM treatments was related to plant life form, and to investigate the longevity of potential bioherbicidal compounds in the soil.

# MATERIALS AND METHODS

## Study Sites and Sample Collection

In the summer of 2011, field studies were established on an organic field at Lewis-Brown Horticulture Research Farm, Oregon State University (Oregon, USA; 43º 33′ 24″ N, 123º 13′ 7″ W). Soil at the experimental site was classified as a Chehalis silty-clay loam (fine-silty, Ultic Haploxerolls) with an organic content of 4.8%, pH of 6.1, a cation exchange capacity of 29.9 $cmol_c$ $kg^{-1}$, and 40-50% clay. Mineral nitrogen in the soil was 4 mg $kg^{-1}$ soil for nitrate-nitrogen and 2 mg $kg^{-1}$ soil for ammonium-nitrogen. Five soil core samples (5.2 cm diameter x 7.5 cm depth) were taken randomly, dried at 70 ºC, and weighed to calculate soil bulk density which was 1.36±0.011 g $cm^{-3}$. Two studies were conducted from June 15 to August 18 for Experiment 1, and from July 22 to September 16 for Experiment 2. Each study was structured as a randomized complete block design with four replications of seven treatments: a control (no MSM amendment), three MSM concentrations by weight (3, 5, and 7%) of two forms of MSM (non-activated MSM and activated MSM).

MSM[1] and ground meadowfoam seeds were passed through a 1 mm-sieve before use. MSM added to each plot was calculated on a meal weight and air-dry soil weight for a 3-cm depth of incorporation. Soil weight was calculated based on bulk density (Rice et al. 2007). MSM was applied by weight at 3 rates per square meter: 3% (1.22 kg), 5% (2.04 kg), and 7% (2.86 kg). Non-activated MSM contained only sieved MSM. Activated MSM was prepared by adding 1% by weight of freshly ground meadowfoam seeds to MSM in order to provide active myrosinase (Stevens et al. 2009).

The plot size was 1.6 $m^2$ with 1.07 m border between plots and 1.07 m border around the entire site. Plots were plowed, tilled, and hand-weeded before seed meal incorporation. Seed meal treatments were applied by spreading and incorporating to 3-cm using a garden rake. Immediately after MSM application, 19 mm of water was applied by overhead irrigation to the plot area. A drip irrigation system of 3 lines of drip tapes[2] per plot with 20-cm emitter spacing was used. The irrigation rate was 500 L per 100 m length per hr, at 55 kPa operating pressure. Drip irrigation was started one day prior to lettuce transplanting. The irrigation schedule was 20 min $d^{-1}$ for one week after lettuce transplanting, and then 15 min $d^{-1}$, two times a week until weed removal. The air temperature was recorded for 28 d after MSM incorporation (Figure 3.1).

Leaf lettuce[3] (*Lactuca sativa* L. 'Black Seeded Simpson'), was chosen as a short season crop for the studies. The variety has a 45-day maturity and was recommended for transplanting in Oregon (Hemphill 2010). Lettuce seeds were sown in propagation trays[4] in the greenhouse with 25/20 ºC day/night temperature and a 14 hr photoperiod. Lettuce seedlings (26 d old) were transplanted in plots 7 days after meal incorporation (DAI) to prevent injury from MSM. Nine lettuce seedlings were transplanted in each of 3 rows with 30 cm between-row-spacing and 15 cm in-row-spacing for a total of 27 plants per plot.

Five lettuce plants were harvested for above ground biomass from the middle row of each plot 21 d after transplanting. Weeds were sampled from 1.1 $m^2$ in each plot. Weed species were identified, counted, and harvested for above ground biomass 64 DAI. Lettuce and weed biomass were dried at 60 ºC for 72 hr, and weighed. Ground lettuce

leaf tissue was used to measure total nitrogen (N) by dry combustion (Nelson and Sommers, 1996) using a carbon, nitrogen, and sulfur analyzer[5].

**Glucolimnanthin and Its Breakdown Products**

Soil samples were taken from 0.5 $m^2$ area of each plot using a 2.5 cm diameter soil core. Three soil cores from each plot were sampled at 0-15 cm depth. Soil samples were composited to form one representative sample for each plot and kept in a cool container during transport to the laboratory. Subsamples (approximately 20 g moist soil) were weighed, placed in aluminum cups, dried at 120 ºC for 2 hr, and reweighed to determine soil moisture content.

The extraction method for extracting glucosinolate and its hydrolysis compounds from the soil was developed by modifying the method of Stevens et al. (2009). Glucolimnanthin (CAS 111810-95-8, S-(β-D-glucopyranosyl)-(Z)-2-(3-methoxyphenyl)-N-(sulfooxy)ethanimidothioic acid potassium salt) was extracted from meadowfoam seed meal. 3-Methoxybenzyl isothiocyanate[6] (isothiocyanate), 3-methoxyphenylacetonitrile[7] (nitrile), and 3-methoxyphenylacetic acid[8] were used as standards for GLN and GBP analyses.

Soil samples (2 g equivalent dry weight) were placed into a 15 ml centrifuge tube. Each incubated soil tube received 6 ml of 70% methanol. The tube was shaken, sonicated for 10 min, and allowed to stand for 60 min. The mixture was centrifuged for 5 min at 3,000 rpm. The supernatant was centrifuged for 10 min at 13,000 rpm. The methanol concentration in the supernatant was increased to 90% to prevent further enzymatic degradation of GLN. The analyses of GLN and GBPs were performed by using high

performance liquid chromatography (HPLC) as described by Stevens et al. (2009). The injection volume was 30 µl. A Waters 2996 photodiode array detector (210 to 500 nm) at 274 nm was used to calculate peak areas for all compounds. Analyte concentrations were determined from calibration curves constructed for each analyte using the external standard method. The GLN and GBPs were quantified on 3, 6, 9, and 12 DAI. In Experiment 2, additional GLN and GBP analyses were performed at 1 DAI.

**Post-harvest Soil Nitrate-nitrogen**

On the day of lettuce harvest (28 DAI), soil samples were collected within 0.5 $m^2$ area of each plot using a 2.5 cm diameter soil core. Six soil cores were collected at a 15-cm depth from each plot and composited to form one representative sample per plot. Soil samples were kept in a cool container during transport to the laboratory. Concentrations of nitrate-nitrogen were determined by extraction with 2 N KCl (Horneck et al. 1989).

**Plant and Soil Data Analyses**

Relative weed emergence was calculated as percent of weed emergence in the untreated control plot. Total lettuce nitrogen was calculated by multiplying lettuce biomass per gram by nitrogen concentration of leaf tissue. Lettuce and weed biomass, lettuce nitrogen, total nitrogen concentration, and GLN and GBP concentrations were analyzed using one-way analysis of variance (one-way ANOVA) with means separated using a least significant difference (LSD) test at a 0.05 level PROC GLM in SAS v. 9.2[9]. Concentrations of nitrate-nitrogen were subjected to log transformation to stabilize

variances. Correlation analysis was performed on concentrations of nitrate-nitrogen and weed biomass using a Pearson correlation test PROC CORR in SAS v. 9.2.

**Weed Community Data Analyses**

Weed species biomass in each treatment plot was subjected to permutation-based multivariate analysis of variance (PerMANOVA) and nonmetric multidimensional scaling (NMS) using the multivariate statistical software package PC-ORD v. 6.12[10] (McCune and Mefford 2011).

*Data preparation*. The main species matrix consisted of weed biomass measured across the 28 sample plots in each experiment. There were 41 and 37 weed species identified in Experiment 1 and 2, respectively (see Appendix A.1). The occurrence of individual weed species across sample units ranged from 1 to 28 in the two studies. Rare species were removed to enhance the detection of relationships between community composition and environmental factors (McCune and Grace 2002). In the data sets, three weed species in Experiment 1 and five weed species in Experiment 2 were removed because the species occurred only once. The data without rare species, 38 species in Experiment 1 and 32 species in Experiment 2, were subjected to cube root transformation in order to compress the larger values and widen the distances among the lower values. This transformation provided a more even distribution of the data along the biomass scale, as compared to non-transformed data (data not shown). The transformed data with rare species removed were used in all subsequent analyses.

*Weed community composition.* PerMANOVA was performed to test the null hypothesis of no difference in weed community and plant life form patterns due to MSM treatments. PerMANOVA is a non-parametric procedure for testing group differences analogous to parametric ANOVA, which evaluates significance using a pseudo-*F*-ratio and a permutation test (Anderson 2001; McCune and Grace 2002).

PerMANOVA was used to evaluate the differences in various plant life forms in response to treatments using the life form score matrices (procedures as further described below). When including control plots with MSM treatment plots to evaluate the effects of each treatment on weed biomass or life form, a randomized complete block design was used. A two-way factorial design was used to compare MSM concentrations and MSM forms. Sørensen distance was selected for its tendency to retain sensitivity in heterogeneous data sets and because it gives less weight to outliers (McCune and Mefford 2011).

*Weed community patterns and relationships.* An ordination of the multidimensional species data was used to investigate patterns of weed species biomass changes in relation to the MSM treatments using NMS (Kruskal 1964; Mather 1976). In plant life form variables, weed species were sorted by cotyledon classification (dicot and monocot) and life form (annual, biennial, and perennial) based on species-specific information obtained from the PLANTS database (USDA 2012). Absence or presence of a plant life form (annual, biennial, perennial, dicot, and monocot) for each species was converted to a binary format (0 or 1). Some species had more than one possible life cycle based on environmental conditions. For these species, life cycles were defined using the common

categories of each plant in the Willamette Valley (C. Mallory-Smith, personal communication). Species matrices with rare species removed and transformation as previously described were multiplied by each transposed life form matrix in the same experiment to obtain a life form score matrix for each experiment (28 plots x 5 life forms). NMS ordinations of sample units (plots) in species space used autopilot, slow and thorough, tie penalizing, and Sørensen distance for all runs. The plant life form variables were plotted as vectors onto NMS joint plots to indicate direction and strength of the relationships between plant life form variables with sample unit (plot) ordination scores. The strength of these vectors was determined using the Mantel's randomization (Monte-Carlo) test (Mantel 1967; McCune and Mefford 2011) and Euclidean distance (McCune and Grace 2002).

# RESULTS

The patterns of lettuce growth, soil nitrate-nitrogen, weed emergence and growth, weed community composition, and total concentration of GLN and GBPs varied between the two experiments. The air temperature between the two experiments was dissimilar and fluctuated (Figure 3.1). Therefore, statistical analyses were performed by experiment.

## Effect of Meadowfoam Seed Meal on Lettuce

Average lettuce biomass was about 6 to 9 times greater in MSM treatments than in the untreated control in both experiments (Table 3.1). Lettuce leaves were dark-green in the MSM treatments, and yellow to light green in the untreated control (personal observation). Mean nitrogen in lettuce tissue of MSM treatments was more than 34% and 24% greater than the untreated control for Experiment 1 and 2, respectively. Total plant nitrogen was up to 17- and 10-fold greater than the untreated control for Experiment 1 and 2, respectively. More lettuce biomass was produced in Experiment 1 (0.5 g plant$^{-1}$ for untreated control and ranged from 3.9 to 4.6 g plant$^{-1}$ for MSM treatments) compared to Experiment 2 (0.4 g plant$^{-1}$ for untreated control and ranged from 2.5 to 2.8 g plant$^{-1}$ for MSM treatments) ($p < 0.001$, data not shown).

## Effect of Meadowfoam Seed Meal on Weed

Average total number of emerged weeds was less in MSM amended compared to non-amended plots (Figure 3.2). Weed emergence was not different for MSM forms ($p = 0.74$ for Experiment 1 and $p = 0.83$ for Experiment 2) but was for MSM concentrations

($p$ = 0.004 for Experiment 1 and $p$ < 0.001 for Experiment 2). In Experiment 1, average weed emergence was 57%, 43%, and 32% of the untreated control for 3%, 5%, and 7% MSM, respectively. In Experiment 2, average weed emergence was 72%, 46%, and 46% of the untreated control for 3%, 5%, and 7% MSM, respectively.

Total weed biomass was not different across MSM treatments and the control for both experiments ($p$ = 0.37 for Experiment 1; $p$ = 0.06 for Experiment 2) (Figure 3.3). However, there was more weed biomass in MSM amended treatments compared to the non-amended control. Biomass of weed species was grouped by life form within treatments (see Appendix B.1 for life form and frequency of weed species). Annual weeds were dominant in all treatments of both experiments. In Experiment 1, annual monocots and dicots accounted for 55% and 35%, respectively, of total weed biomass across treatments. Life form compositions between non-amended and 7% activated MSM treatments were not different ($p$ = 0.1). In Experiment 2, annual monocots and dicots were 50% and 45%, respectively, of total weed biomass across treatments. There was a positive correlation between the log transformed soil nitrate-nitrogen content and weed biomass ($r$ = 0.81, $p$ < 0.001 for Experiment 1; $r$ = 0.47, $p$ = 0.013 for Experiment 2) (Figure 3.4).

**Effect of Meadowfoam Seed Meal on Weed Community**

No differences in weed community structure in relation to different concentrations and forms of MSM were found either with (RCB design) or without the untreated control (a two-way factorial design) (Table 3.2). In analyses using plant life form scores for annual, biennial, perennial, dicot, and monocot across experiment plots, weed

communities differed among MSM concentrations in both experiments (PerMANOVA, two-way factorial: $F = 3.71$, $p = 0.02$ for Experiment 1; $F = 2.61$, $p = 0.06$ for Experiment 2) but not among MSM forms. However, plant life forms varied among blocks in Experiment 2 (PerMANOVA, RCB: $F = 2.26$, $p = 0.05$).

**Patterns and Relationship of Weed Community**

In both experiments, the multivariate NMS analysis recommended a three-dimensional solution. Comparisons of the stress values after 250 runs with real data and 250 runs with randomized data using a Monte Carlo randomization test showed that the three-axis solution was stronger than expected by chance ($p = 0.004$ for Experiment 1 and $p = 0.048$ for Experiment 2).

Axis numbers in NMS are arbitrary (McCune and Grace 2002). Rotation was performed to maximize the strength of the relationship between plant life form variables and ordination axes by alignment with axis 1 for both experiments (Figure 3.5). In Experiment 1, the pair of axes 1 and 3 was selected for the ordination alignment. The three dimensions accounted for a total of 90.1% of the total variation with axes 1, 2, and 3 contributing 49.8%, 25.4%, and 14.9% of the total variance, respectively. In Experiment 2, the three dimensions accounted for a total of 87.8% of the total variation with axes 1, 2, and 3 contributing 48.0%, 25.7%, and 14.1% of the total variance, respectively.

Weed species composition from different soil amendment materials had patterns of plant distribution grouped by plant life forms on the basis of NMS of cube root transformed weed biomass data (Figure 3.5). Correlations of plant life forms were

strongest with axis 1 in both experiments (Experiment 1: $r$ = -0.86 for annual; $r$ = -0.64 for monocot; $r$ = 0.67 for perennial and Experiment 2: $r$ = -0.56 for monocot; $r$ = 0.53 for biennial; $r$ = 0.56 for dicot; $r$ = 0.56 for perennial) with less or no strong correlation on axis 2 or 3. Therefore, results and discussion of weed species correlations with plant life forms and amendment materials were limited to axis 1. Correlated weed species with axis 1 greater than the absolute value of 0.5 are shown in Table 3.3 (see Appendix B.2 for more information). In both experiments, ordination plots of 3% MSM were mainly on the left of the ordination diagram, while ordination plots of a non-amended were on the right of the ordination diagram (Figure 3.5). These opposite distributions indicated dissimilarity of weed species composition between 3% MSM-amended and non-amended plots. Vectors in Figure 3.5 show the direction and strength of the relationships between plant life form variables and the ordination. In both experiments, plant life form vectors (monocot and perennial) were related with ordination axes ($p < 0.05$). Those vectors were aligned with axis 1 but had opposite trajectories indicating increasing monocots in concert with decreasing perennial plants.

In relation to species composition (Table 3.3; Appendix B.2), the 3% and 5% MSM amended treatments in Experiment 1 were dominated by annual and monocot plants such as barnyardgrass (*Echinochloa crus-galli* (L.) Medik.) and common purslane (*Portulaca oleracea* L.) which were strongly, negatively associated with axis 1 ($r$ = -0.78 and -0.78, respectively). The ordination showed that the vectors between annual monocot and perennial plant life forms had opposite trajectories, indicating increasing annual monocots in concert with decreasing perennial species.

In Experiment 2, the vectors between monocot species which pointed toward MSM amended plots were opposite to a group of annual, perennial, biennial, and dicot vectors which pointed toward non-amended plots. In Experiment 2, the 3%, 5%, and 7% MSM amended treatments tended to be dominated by monocot plants such as barnyardgrass and wild garlic (*Allium vineale* L.) which were strongly, negatively associated with axis 1 ($r$ = -0.80 and -0.64, respectively). Plant life forms of biennial, perennial, or dicot were dominant in most of non-amended plots. The dominant species included Canada bluegrass (*Poa compressa* L.) ($r$ = 0.66), black nightshade (*Solanum nigrum* L.) ($r$ = 0.54), and black medic (*Medicago lupulina* L.) ($r$ = 0.53).

**Glucolimnanthin and Its Breakdown Products**

In Experiment 1, concentrations of GLN and GBPs varied across MSM concentrations 3 DAI (Figure 3.6). GLN was not detected on 6 DAI, while isothiocyanate concentration was detected only with 7% activated MSM. Nitrile and MPAA were detected on 6 DAI in 7% activated MSM and on 9 DAI in MSM-amended treatments, respectively (data not shown). On 3 DAI, the total concentrations of GLN and GBPs across MSM treatments differed ($p < 0.001$). The 7% activated MSM had at least 46% more GLN and GBP concentrations than other MSM treatments. The difference in GLN and GBP concentrations was detected among the three concentrations of MSM ($p < 0.001$) but not among the forms ($p = 0.29$). MSM at 7% concentration, regardless of form, had greater levels of GLN and GBPs.

In Experiment 2, concentrations of GLN and GBPs were measured on 1 and 3 DAI (Figure 3.7). Isothiocyanate concentration was detected on 1 DAI in the 5% and 7%

activated MSM (Figure 3.7A). GLN and nitrile concentrations were detected 3 DAI in 7% activated MSM, whereas MPAA concentration was detected 6 DAI in all MSM-amended treatments (data not shown). On 1 DAI, 3% non-activated and activated MSM treatments had at least 60% less GLN and GBPs compared to other treatments. GLN and GBPs differed across MSM concentrations ($p < 0.001$) but not among forms ($p = 0.33$). On 3 DAI, no GLN or isothiocyanate was found (Figure 3.7B). Nitrile was present only in 5% and 7% activated MSM. GLN and GBP concentrations differed across MSM concentrations ($p = 0.006$), but not MSM forms ($p = 0.98$).

# DISCUSSION

Rice et al. (2007) suggested using transplants for crop safety in the field with Brassicaceae meal amendments. In our studies, no injury was observed in transplanted lettuce 7 DAI. The safe planting date for lettuce was in agreement with greenhouse studies (see Chapter 2). Lettuce biomass and average lettuce tissue nitrogen content were greater in MSM treatments compared to the untreated control. Nitrogen supplied by MSM was a factor resulting in the greater lettuce biomass. The soil test for mineral nitrogen prior to the field study confirmed that there was a typical level of ammonium-nitrogen (observed 2 mg $kg^{-1}$ soil) and a low level of nitrate-nitrogen (observed 4 mg $kg^{-1}$ soil), categorized using the soil test interpretation guide by Marx et al. (1999). However, lettuce biomass was not different across different MSM concentrations and forms. Johnson-Maynard et al. (2005) reported lettuce biomass and lettuce tissue nitrogen collected from soil amended with different concentrations of brown mustard (*Brassica juncea* L.) meal, rapeseed (*B. napus* L.) meal, and yellow mustard (*Sinapis alba* L.) meal. Greater lettuce biomass was found with increasing meal concentrations of rapeseed and brown mustard but greater nitrogen content was only found with increasing meal concentrations of brown mustard amendment.

In general, lettuce biomass in Experiment 1 was greater than in Experiment 2. This result was likely due to the differences in environmental conditions such as temperature and moisture. Experiment 1, started in early summer, had a lower average temperature during the growing season compared to Experiment 2 (Table 3.1). Leaf lettuce establishes well in a temperature range between 16 and 18 ºC, with frequent

irrigation, and plant available nitrogen between 168 to 196 kg $ha^{-1}$ (Hemphill 2010). Nitrate and ammonium nitrogen levels depend on environmental conditions such as temperature and moisture. Over irrigation or heavy rainfall causes the loss of nitrate-nitrogen by leaching while ammonium-nitrogen can be converted to nitrate-nitrogen (Marx et al. 1999).

Post-harvest soil nitrate represents the quantity of plant-available nitrogen present in the nitrate form which was not utilized by the previous crop (Sullivan and Cogger 2003). Weed biomass and soil nitrate contents were low in non-amended treatment plots. Weed biomass was correlated with soil nitrate concentrations in both experiments. Greater soil nitrate levels correlated with greater weed biomass in MSM-amended plots. The excessive nitrate-nitrogen content in the soil from MSM application was likely utilized by emerged weeds.

After lettuce was harvested, the plots were retained and irrigated for an additional 28 d to evaluate the effect of MSM on weeds over a longer period of time. The bioherbicide effect of MSM on weed emergence and growth was short-lived but the fertilizer effect in the MSM amended area remained. The plot area after lettuce harvest provided space for growth of the later emerging weeds. In addition to space, plant available nutrients, especially nitrogen, supported the growth of emerged weeds in MSM-amended plots. Rice et al. (2007) observed similar results with a brown mustard meal application. After the herbicidal effect was gone, the meal promoted the growth of late season redroot pigweed (*Amaranthus retroflexus* L.).

MSM concentrations influenced weed emergence, growth, and community composition more than form of MSM. No difference in number of emerged weeds was

detected between forms with the same concentration of MSM. At higher concentrations (5 and 7% MSM), the bioherbicide effect was greater than at the lower concentration (3% MSM). Lack of a detectable difference in total weed biomass across non-amended and MSM-amended treatments was possibly due to high variations in weed biomass among plots within the same treatment. Weed species with an annual plant life form were dominant in MSM-amended treatments and may possibly utilize more plant-available nutrients than perennial species, especially nitrogen from MSM. Annual *Bromus* species had more efficient uptake of nitrate-nitrogen, but the perennial *Bromus* species survived better when the nitrogen supply was scarce (Muller and Garnier 1990). The same trends were observed between annual and perennial dicots of the genus *Polygonum* (Zangerl and Bazzaz 1983).

Total weed biomass analyzed using the community composition method confirmed the differences among plant life forms in response to MSM concentrations. The plots with greater levels of annual or monocot species such as barnyardgrass, common purslane, and wild garlic, had fewer perennial plants compared to the non-amended treatment. Fewer perennial and biennial dicots in MSM treatments may be due to either lower competitive ability of these species or herbicidal selectivity of MSM. Gaudet and Keddy (1995) and Rösch et al. (1997) reported that larger plants were competitive for light when grown under fertile condition. MSM suppression of weed emergence and growth did not show any promising selectivity for a particular weed species. Herbicidal selectivity of MSM needs further investigation. In previous studies, MSM inhibited seed emergence and growth of an annual monocot, downy brome

(*Bromus tectorum* L.) (Machado 2007; Stevens et al. 2009) and an annual dicot, velvetleaf (*Abutilon theophrasti* Medik.) (Vaughn et al. 1996).

In both experiments, GLN and GBP concentrations were not different between activation forms, but there were differences among concentrations. MSM concentrations of 5% and 7% had higher GLN and GBP concentrations than 3% MSM. However, compound composition varied across MSM forms. Isothiocyanate which had the most potent herbicidal activity among GBPs (see Chapter 2) was detected in higher concentrations of activated MSM. With soil incorporation of 3% activated MSM, isothiocyanate may have degraded before 24 hr. In the activation process, GLN is hydrolyzed by the addition of myrosinase from freshly ground meadowfoam seeds and then isothiocynate is produced (Stevens et al. 2009). Isothiocyanate has a short half-life in soil from 20 to 60 hr depending on soil type (Borek et al. 1995). When observed in a closed container with 3% activated MSM amended soil and 38% soil moisture, isothiocyanate was metabolized within 72 hr (see Chapter 2). Borek et al. (1995) reported that the degradation rate of isothiocyanate increased with lower soil moisture, higher temperature, and higher concentration of soil organic carbon, whereas the degradation rate of allynitrile increased with higher soil moisture, lower temperature, and higher concentration of soil carbon.

Lettuce growth, nitrogen content in lettuce tissue, weed emergence and growth, and weed community composition were not different for forms (activated and non-activated MSM) but were for concentrations (3, 5, and 7%). Morra and Kirkegaard (2002) noted that an increase in glucosinolate-containing tissue concentrations did not result in a proportional increase in soil-extractable ITC. The most limiting factor for ITC

concentration was not the glucosinolate concentration, but the release rate of glucosinolate from plant tissue to the soil. Previously, the recommended activation method for MSM was to ferment MSM by mixing ground meadowfoam seeds into MSM, adding water, and allowing it stand overnight before application (Stevens et al. 2009). However, the wet form was not convenient for large-scale field application. Therefore, in this study we prepared the activated MSM without prior fermentation or adding water in order to initiate an application method of MSM at a field level.

MSM has both bioherbicidal and fertilizer effects. In order to utilize the herbicidal activity of MSM, the application should be between 3% and 7% by weight and incorporated close to transplanted crops (such as band application) to provide weed suppression at the beginning of crop establishment and avoid too much fertilizer for later emerging weeds. The activated MSM did not give the same results for weed control compared to previous studies in the growth chamber and greenhouse (see Chapter 2). This difference may be due to the fast dissipation of GLN and GBPs in the field by leaching or degradation by soil microorganisms. Under soil conditions of high soil organic carbon, bioactivity of isothiocyanate also may decrease due to the reaction between functional groups of isothiocyanate and nucleophilic functional groups in soil organic carbon (Borek et al. 1995). The optimization ratio between ground meadowfoam seed to MSM needs further investigation in order to use MSM more efficiently. Morra and Kirkegaard (2002) suggested increasing bioherbicide activity by disruption of glucosinolate-containing tissues in order to provide greater contact between glucosinolate and myrosinase.

Although the MSM treatments were weakly related to weed community composition, there was a relationship between MSM concentrations and plant life forms. MSM treatment effects on specific weed species should be further investigated, because there was evidence that some weed species (barnyardgrass, common purslane, and wild garlic) were more abundant in MSM treatments, while some species (such as Canada bluegrass, black medic, and black nightshade) were not.

The non-amended plots in this study were considered to be a pseudo-control because there were no amendment materials, while MSM amended plots resulted in additional organic inputs. The results of this study cannot be extended to the potential effects of MSM on weed community composition in other areas. In ecological communities, other factors to be considered include weed seed banks, soil types, soil moisture contents, microorganism communities, and also environmental conditions across longer timespans.

## SOURCES OF MATERIALS

[1] Meadowfoam seed meal, Natural Plant Products, Inc., 707 13th St. SE, Suite 275, Salem, OR 97301.

[2] Drip tape, Ro-drip; John Deere, Moline, IL 61265.

[3] Leaf lettuce 'Black Seeded Simpson', certified organic seed, Planation Products Inc., 202 S. Washington St., Norton, MA 02766.

[4] Propagation tray, Landmark Plastic Corporation, Akron, OH 44306.

[5] Carbon, nitrogen, and sulfur analyzer, Leco CNS-2000, Leco Corporation, 3000 Lakeview Ave., St. Joseph, MI 49085.

[6] 3-Methoxybenzyl isothiocyanate, Oakwood Products, West Columbia, SC 29172

[7] 3-Methoxyphenylacetonitrile, Sigma-Aldrich Chemicals, St. Louis, MO 63178.

[8] 3-Methoxyphenylacetic acid, TCI America, Portland, OR 97203.

[9] SAS version 9.2, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513.

[10] PC-ORD 6.12, MjM Software Design, PO Box 129, Gleneden Beach, OR 97388.

## LITERATURE CITED

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral. Ecol. 26: 32-46.

Bartelt, R. J. and K. L. Mikolajczak. 1989. Toxicity of compounds derived from *Limnanthes alba* seed to fall armyworm (Lepidoptera: Noctuidae) and European corn borer (Lepidoptera: Pyralidae) Larvae. J. Econ. Entomol. 82: 1054-1060.

Borek, V., M. J. Morra, P. D. Brown, and J. P. McCaffrey. 1995. Transformation of the glucosinolate-derived allelochemicals allylisothiocyanate and allylnitrile in soil. J. Agric. Food Chem. 43: 1935-1940.

Ehrensing, D. T., G. D. Jolliff, J. M. Crane, and R. S. Karow. 1997. Growing meadowfoam in the Willamette Valley. Oregon State University Extension Service. EM 8567.

Gaudet, C. L. and P. A. Keddy. 1995. Competitive performance and species distribution in shortline plant communities: a comparative approach. Ecol. 76: 280-291.

Gimsing, A. and J. Kirkegaard. 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochem. Rev. 8: 299-310.

Hemphill, D. 2010. Oregon vegetables: lettuce. Available at http://horticulture.oregonstate.edu/content/lettuce-0. Access: May 1, 2011.

Horneck, D. A., J. M. Hart, K. Topper, and B. Koepsell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Corvallis, OR: Agricultural Experiment Station, SM 89:4. 21 p.

Johnson-Maynard, J. M., Morra, L. Dandurand, C. William, and M. J. Butters. 2005. Brassicaceae seed meal application for weed reduction and improved nitrogen management in organic farming systems. Project report, University of Idaho, Moscow, ID.

Kleiman, R. 1990. Chemistry of new industrial oilseed crops. Pages 198-199 in J. Janick and J. E. Simon, eds. Advances in New Crops. Portland, OR: Timber Press.

Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika 29: 115-129.

Linderman, R. G., E. A. Davis, and C. J. Masters. 2007. Response of conifer seedlings to potting medium amendment with meadowfoam seed meal. Pages 138-142 in J. Janick and A. Whipkey, eds. Issues in New Crops and New Uses. Alexandria, VA: ASHS Press.

Machado, S. 2007. Allelopathic potential of various plant species on downy brome: implications for weed control in wheat production. Agron. J. 99: 127-132.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. Cancer Res. 27: 209-220.

Martens, M-H. and K. Martens. 2002. Organic weed control: cultural and mechanical methods. Acres U.S.A. 32: 1-7.

Marx, E. S., J. Hart, and R. G. Stevens. 1999. Soil test interpretation guide. Oregon State University Extension Service. EC 1478.

Mather, P. M. 1976. Computational methods of multivariate analysis in physical geography. London: John Wiley & Sons. 532 p.

Matthiessen, J. and J. Kirkegaard. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Crit. Rev. Plant Sci. 25: 235-265.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. Gleneden Beach, OR: MjM Software Design. 300 p.

McCune, B. and M. J. Mefford. 2011. PC-ORD. Gleneden Beach, OR: MjM Software Design.

Monaco, T. J., S. C. Weller, and F. M. Ashton. 2002. Weed Science, Principles and Practices. 4th ed. New York: John Wiley and Sons, Inc. 671 p.

Morra, M. J. and J. A. Kirkegaard. 2002. Isothiocyanate release from soil-incorporated *Brassica* tissues. Soil Biol. Biochem. 34: 1683-1690.

Muller, B. and E. Garnier. 1990. Components of relative growth rate and sensitivity to nitrogen availability in annual and perennial species of *Bromus*. Oecologia 84: 513-518.

Nelson, D. W. and L. E. Sommers. 1996. Total carbon, organic carbon, and organic matter. Pages 961-1010 in D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, M. E. Sumner, eds. Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Ser. 5. Madison, WI: SSSA.

Purdy R. H. and C. D. Craig. 1987. Meadowfoam: new source of long-chain fatty acids. J. Am. Oil Chem. Soc. 64: 1493-1498.

Rösch, H., M. W. V. Rooyen, and G. K. Theron. 1997. Predicting competitive interactions between pioneer plant species by using plant traits. J. Veg. Sci. 8: 489-494.

Rice, A. R., J. L. Johnson-Maynard, D. C. Thill, and M. J. Morra. 2007. Vegetable crop emergence and weed control following amendment with different Brassicaceae seed meals. Renew. Agric. Food Syst. 22: 204-212.

Steiner, J. J., G. W. Mueller-Warrant, S. M. Griffith, G. M. Banowetz, and G. W. Whittaker. 2006. Conservation practices in western Oregon perennial grass seed systems: II. meadowfoam rotation crop management. Agron. J. 98: 1501-1509.

Stevens, J. F., R. L., Reed, S. Alber, L. Pritchett, and S. Machado. 2009. Herbicidal activity of glucosinolate degradation products in fermented meadowfoam (*Limnanthes alba*) seed meal. J. Agric. Food Chem. 57: 1821-1826.

Sullivan, D. M. and C. G. Cogger. 2003. Post-harvest soil nitrate testing for manured cropping systems, west of the Cascades. Oregon State University Extension Service. EM 8832-E.

USDA, NRCS. 2012. The PLANTS Database. Available at http://plants.usda.gov. Accessed: February 13, 2012.

VanEtten, C. H. and H. L. Tookey. 1978. Glucosinolates in cruciferous plants. Page 507-520 in R. F. Keeler, K. R. Van Kampen, and L. F. James, eds. Effects of Poisonous Plants on Livestock. New York: Academic Press.

Vaughn, S. F., M. A. Berhow, and B. Tisserat. 2008. Stimulation of plant growth by (3-methoxyphenyl) acetonitrile applied as a foliar spray *in vivo* or as a medium amendment *in vitro*. HortSci. 43: 372-375.

Vaughn, S. F., R. A. Boydston, and C. A. Mallory-Smith. 1996. Isolation and identification of (3-methoxyphenyl) acetonitrile as a phytotoxin from meadowfoam (*Limnanthes alba*) seed meal. J. Chem Ecol. 22: 1939-1949.

Vaughn, S. F., D. E. Palmquist, S. M. Duval, and M. A. Berhow. 2006. Herbicidal activity of glucosinolate-containing seedmeals. Weed Sci. 54: 743-748.

Zangerl, A. R. and F. A. Bazzaz. 1983. Responses of an early and a late successional species of *Polygonum* to variations in resource availability. Oecologia 56: 397-404.

Zasada, I. A., J. E. Weiland, R. L. Reed, and J. F. Stevens. 2012. Activity of meadowfoam (*Limnanthes alba*) seed meal glucolimnanthin degradation products against soilborne pathogens. J. Agric. Food Chem. 60: 339-345.

Table 3.1. Average lettuce biomass, percent of nitrogen in lettuce leaf tissue, and total lettuce nitrogen.

| Expt | Meal[a] | Lettuce biomass[b] | Leaf nitrogen | Total nitrogen |
|---|---|---|---|---|
| | --%-- | ---g plant$^{-1}$--- | ---%--- | ---g--- |
| 1 | 0 | 0.5 (0.05) b | 4.6 (0.01) d | 0.02 (0.003) b |
| | 3N | 3.9 (0.39) a | 7.1 (0.13) abc | 0.28 (0.022) a |
| | 5N | 4.5 (0.42) a | 7.5 (0.18) a | 0.34 (0.025) a |
| | 7N | 4.6 (0.36) a | 7.1 (0.16) bc | 0.33 (0.023) a |
| | 3A | 3.9 (0.28) a | 7.0 (0.10) c | 0.28 (0.020) a |
| | 5A | 4.0 (0.18) a | 7.5 (0.19) ab | 0.30 (0.016) a |
| | 7A | 3.9 (0.40) a | 7.3 (0.06) abc | 0.29 (0.031) a |
| 2 | 0 | 0.4 (0.05) b | 5.1 (0.12) c | 0.02 (0.003) b |
| | 3N | 2.7 (0.35) a | 6.8 (0.13) b | 0.18 (0.025) a |
| | 5N | 2.5 (0.11) a | 7.0 (0.16) ab | 0.17 (0.009) a |
| | 7N | 2.7 (0.23) a | 7.2 (0.18) a | 0.19 (0.020) a |
| | 3A | 2.7 (0.30) a | 6.7 (0.24) b | 0.18 (0.024) a |
| | 5A | 2.7 (0.17) a | 7.2 (0.18) a | 0.20 (0.017) a |
| | 7A | 2.8 (0.18) a | 7.1 (0.37) ab | 0.20 (0.015) a |

[a] Soil incorporation with non-amended (0%) and meadowfoam seed meal containing the three concentrations and two forms. N = non-activated; A = activated.

[b] Data are represented as means with standard errors within parentheses. Different letters within a column in the same experiment indicate significant differences at the 0.05 level using Fisher's LSD.

Table 3.2. Permutation-based multivariate analysis of variance (PerMANOVA) tests for the differences in biomass of weed community and plant life form scores among meadowfoam seed meal treatments.

| Experimental designs | Factors | d.f. | Weed community | | Life form scores[a] | |
|---|---|---|---|---|---|---|
| | | | F-ratio | *p*-value | F-ratio | *p*-value |
| | ----------Experiment 1---------- | | | | | |
| Randomized complete block[b] | Block | 3 | 0.909 | 0.582 | 0.169 | 0.352 |
| | Treatment[c] | 6 | 1.041 | 0.394 | 1.572 | 0.156 |
| Two-way factorial[d] | Concentration | 2 | 1.268 | 0.206 | 2.606 | 0.055 |
| | Form | 1 | 1.150 | 0.293 | 1.580 | 0.208 |
| | Concentration x form | 2 | 0.428 | 0.987 | 0.346 | 0.862 |
| | ----------Experiment 2---------- | | | | | |
| Randomized complete block | Block | 3 | 0.988 | 0.489 | 2.261 | 0.049 |
| | Treatment | 6 | 1.254 | 0.132 | 1.888 | 0.065 |
| Two-way factorial | Concentration | 2 | 1.323 | 0.157 | 3.706 | 0.016 |
| | Form | 1 | 0.544 | 0.877 | -0.023 | 0.983 |
| | Concentration x form | 2 | 0.455 | 0.983 | 0.147 | 0.974 |

[a] Plant life form scores are calculated as the product of the weed species biomass and five plant life forms (annual, biennial, perennial, dicot, and monocot) assigned to each species found in each of the treatment plots.
[b] Data includes weed biomass in non-amended plots.
[c] Treatments containing the three MSM concentrations (3, 5, and 7%), the two MSM forms (activated and non-activated) of each concentration, and the non-amended treatment.
[d] Data excludes weed biomass in non-amended plots.

Table 3.3. Correlation of weed species variables to nonmetric multidimensional scaling ordination for analysis of soil amendment materials in Experiment 1 and 2 (Pearson, $r \geq |0.5|$). See Appendix A.2 for more information.

| Experiment 1: Axis 1 | | Experiment 2: Axis 1 | |
|---|---|---|---|
| Plant life form | $r$[a] | Plant life form | $r$ |
| Annual dicots | | Annual monocots | |
| *Portulaca oleracea* | -0.777 | *Echinochloa crus-galli* | -0.798 |
| Annual monocots | | Perennial monocots | |
| *Echinochloa crus-galli* | -0.761 | *Allium vineale* | -0.642 |
| *Panicum capillare* | 0.739 | *Poa compressa* | 0.661 |
| | | Annual dicots | |
| | | *Medicago lupulina* | 0.529 |
| | | *Solanum nigrum* | 0.542 |
| | | Perennial dicots | |
| | | *Rumex crispus* | 0.515 |

[a] $r$, Pearson's regression coefficient.

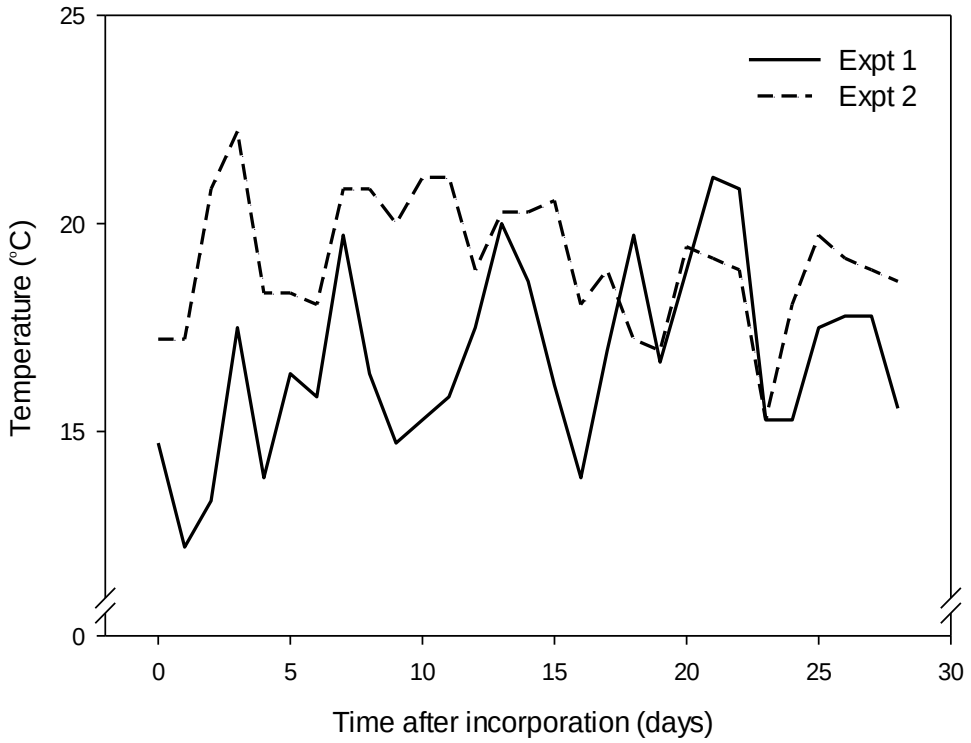



Figure 3.1. Average daily air temperature recorded in 2011 at a weather station located in Hyslop Field Lab, Corvallis, Oregon, approximately 13 km from the study site.

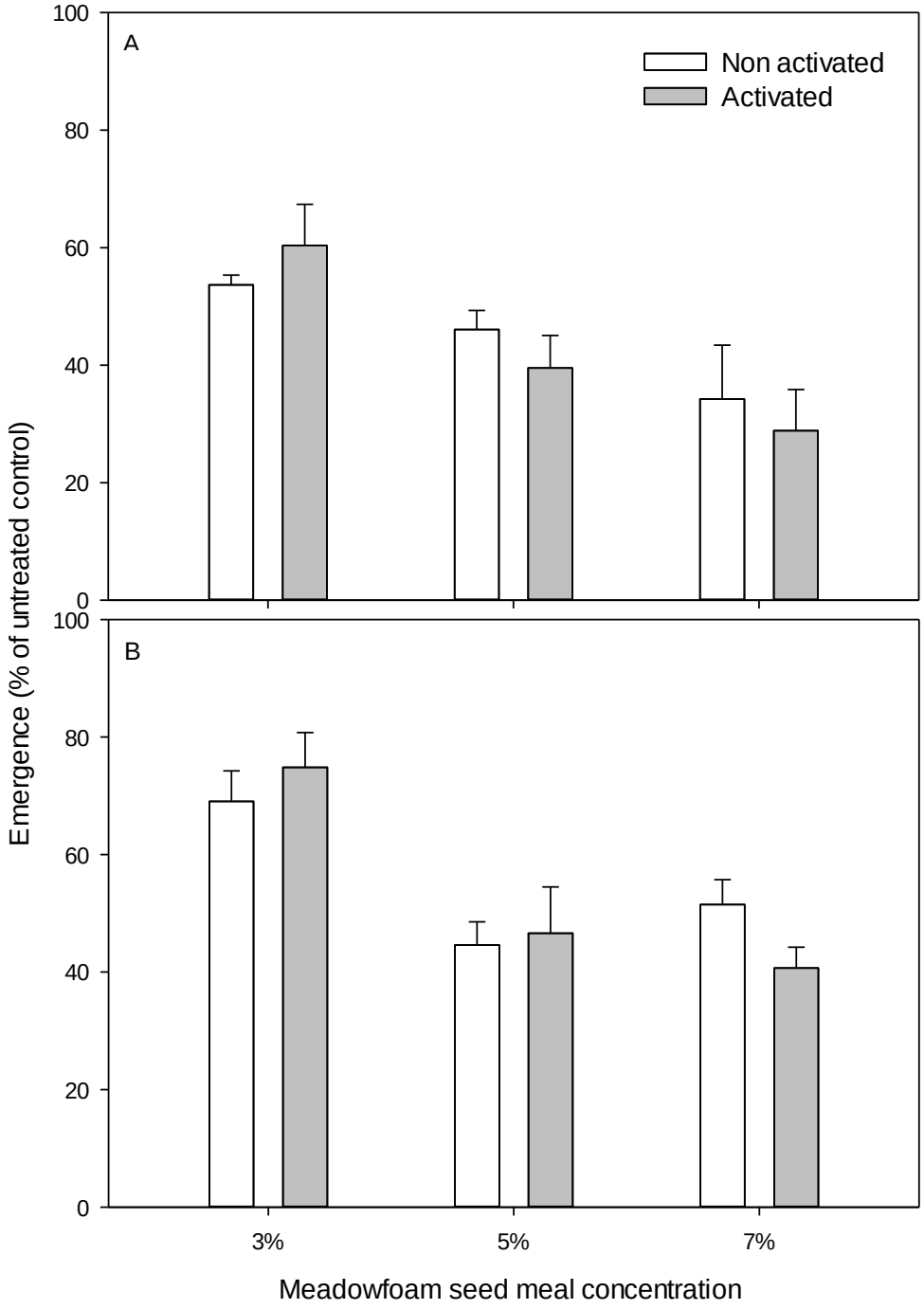



Figure 3.2. Relative weed emergence harvested from 1.1 $m^2$ 56 days after meal incorporation in Experiment 1 (A) and Experiment 2 (B). Bars and error bars represent means and standard errors of sample plots ($n$ = 4).

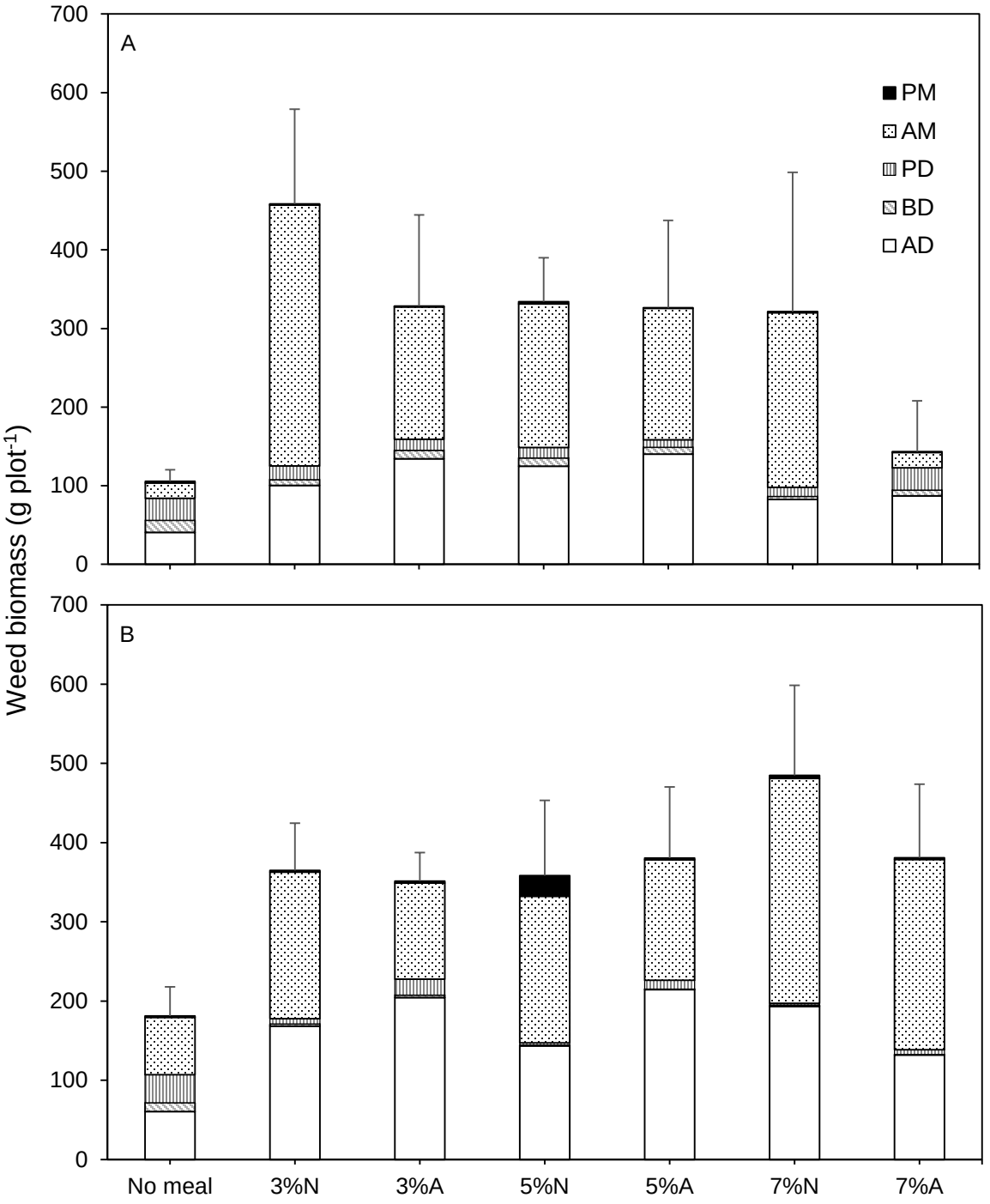



Figure 3.3. Total weed biomass by life forms, harvested from 1.1 $m^2$ 56 days after soil amendment with meadowfoam seed meal containing the three concentrations (3, 5, and 7%) and two forms (N = non-activated; A = activated) in Experiment 1 (A) and Experiment 2 (B). Life form codes: PM = perennial monocot; AM = annual monocot; PD = perennial dicot; BD = biennial dicot; AD = annual dicot. Vertical bars and error bars represent means and standard errors of total weed biomass in each sample plot (*n* = 4).

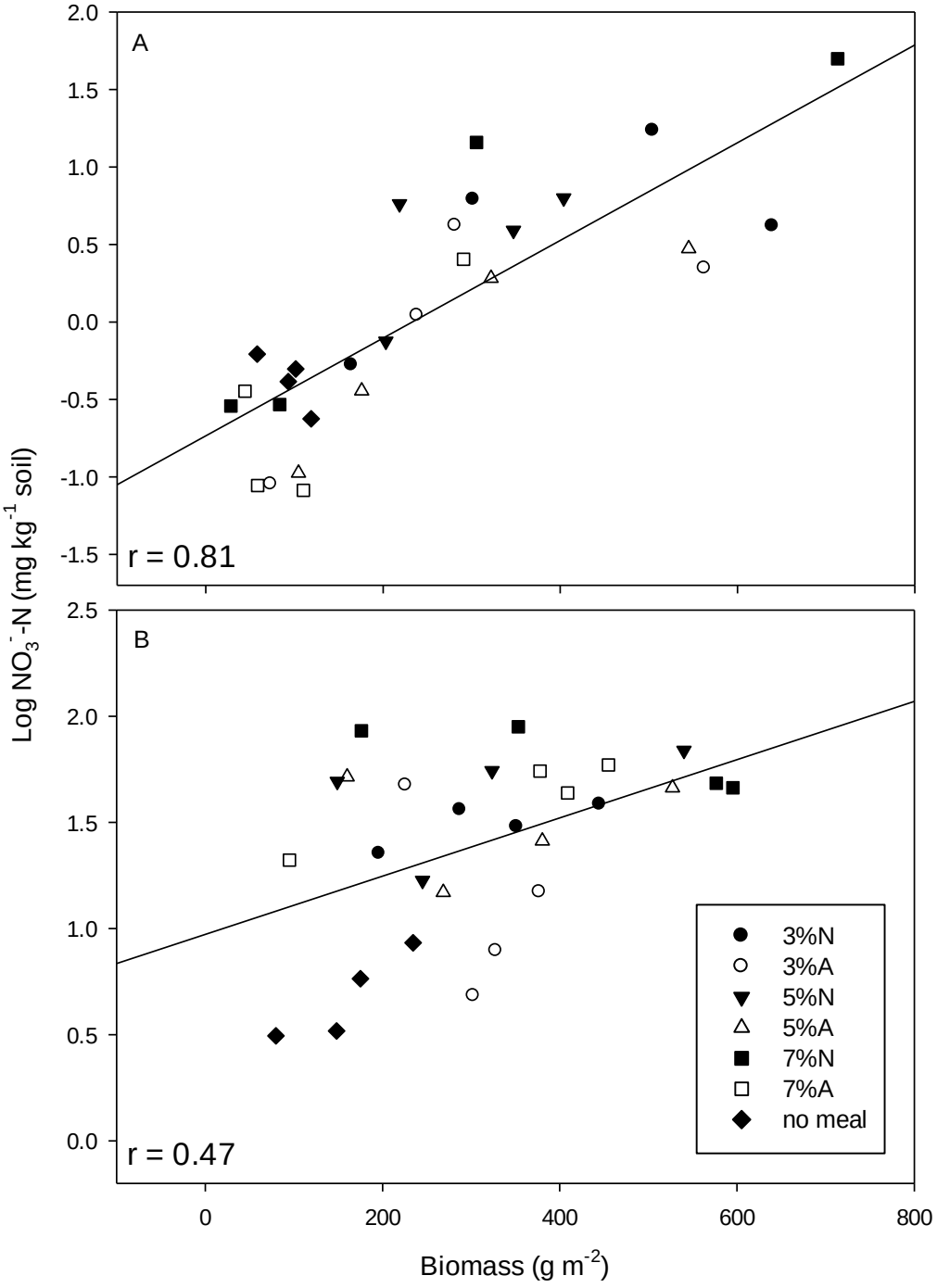



Figure 3.4. Correlation (r) between soil nitrate-nitrogen concentration and weed biomass collected from soil amended with three concentrations (3, 5, and 7%) and two forms (N = non-activated; A = activated) of meadowfoam seed meal in Experiment 1 (A) and Experiment 2 (B).

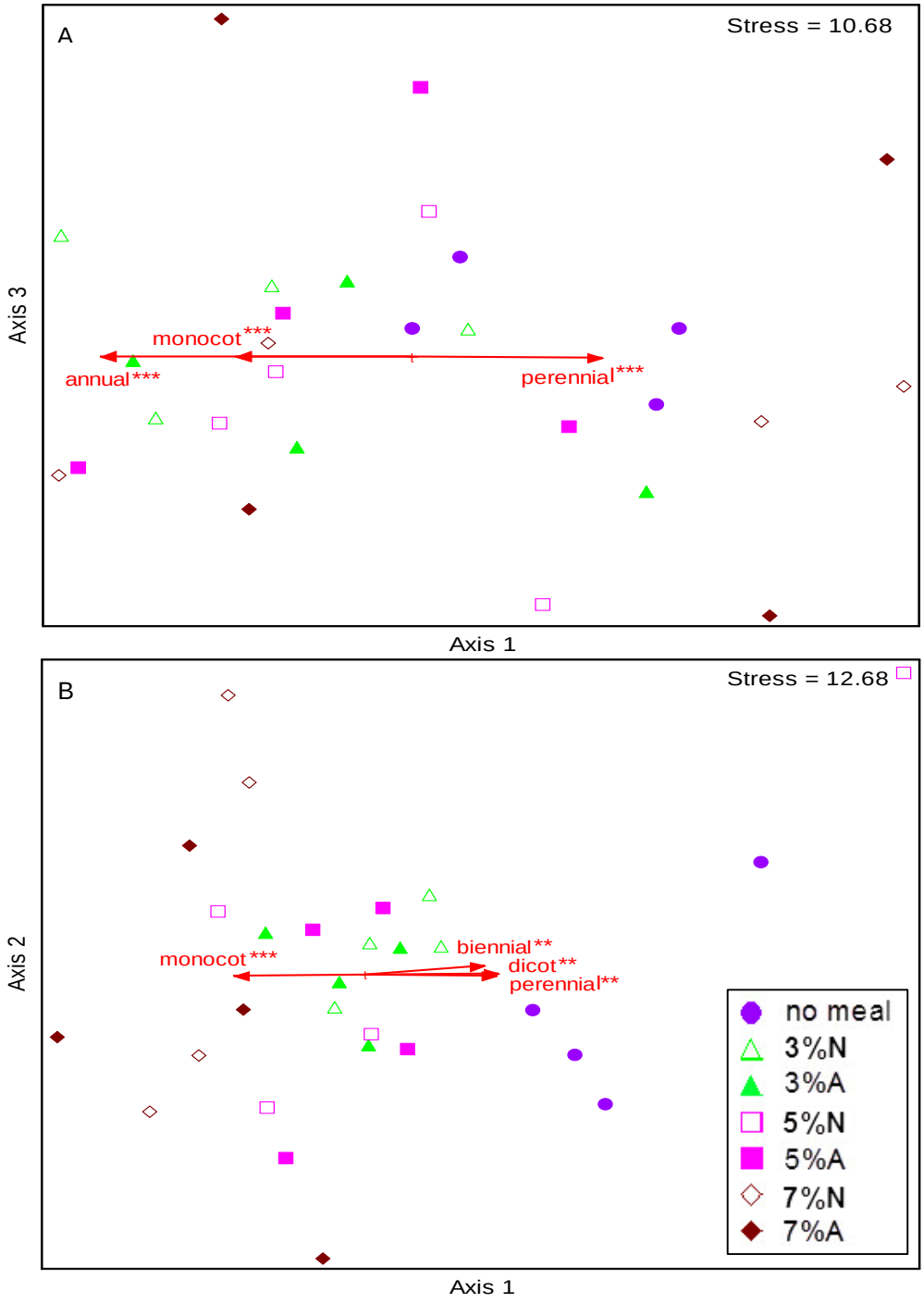



Figure 3.5. Nonmetric multidimensional scaling ordination of the 28 sample plots for Experiment 1 (A) and Experiment 2 (B). Vectors represent the direction and strength of the relationship between plant life form variables and ordination axes. The length of the vector from centroid ($^{+}$) is proportional to the square of the Pearson correlation coefficient; ** = $0.01 < p < 0.05$; *** = $0.001 < p < 0.01$; three concentrations (3, 5, and 7%) and two forms (N = non-activated; A = activated) of meadowfoam seed meal.

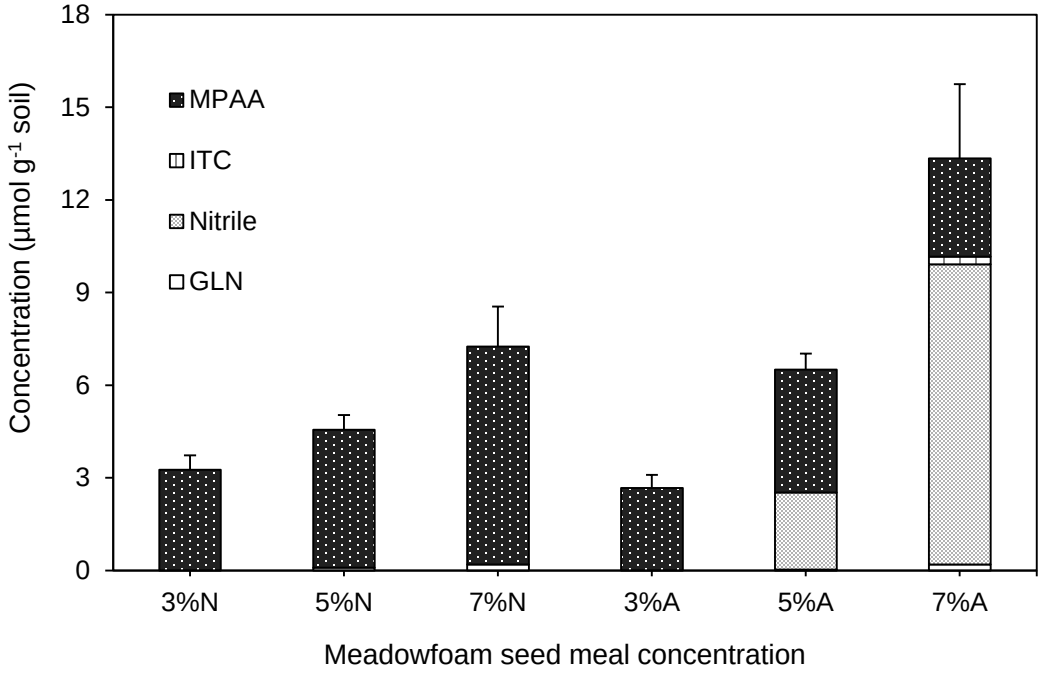



Figure 3.6. Concentrations of glucolimnanthin and its breakdown products in field soil of Experiment 1 amended with three concentrations and two forms of meadowfoam seed meal. Soil was collected 3 days after soil incorporation, at 0-15 cm depth. MPAA = 3-methoxyphenylacetic acid; ITC = isothiocyanate; NIT = nitrile; GLN = glucolimnanthin; N = non-activated; A = activated.

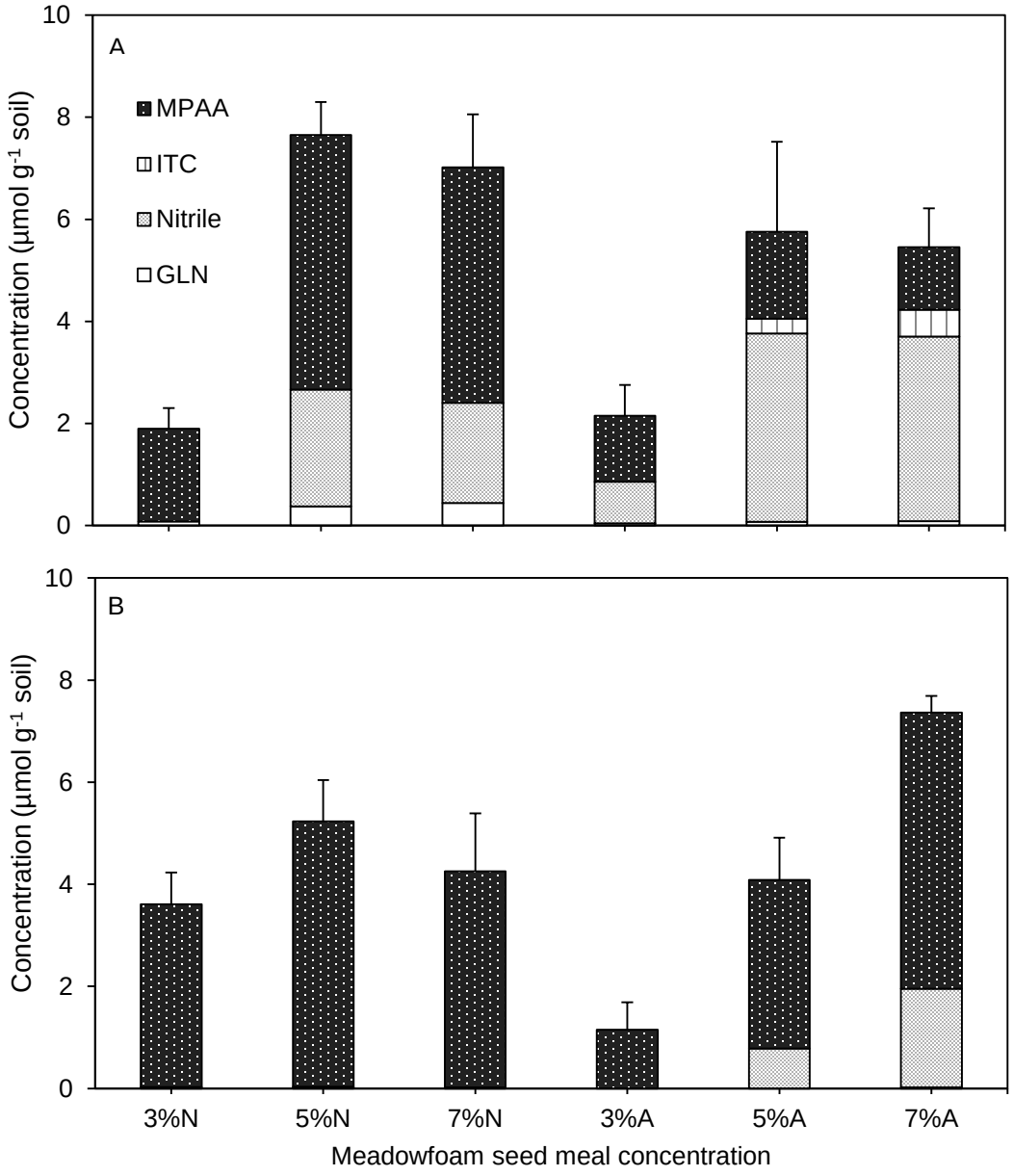



Figure 3.7. Concentrations of glucolimnanthin and its breakdown products in field soil of Experiment 2 amended with three concentrations and two forms of meadowfoam seed meal. Soil was collected 1 day (A) and 3 days (B) after soil incorporation, at 0-15 cm depth. MPAA = 3-methoxyphenylacetic acid; ITC = isothiocyanate; NIT = nitrile; GLN = glucolimnanthin; N = non-activated; A = activated.

# CHAPTER 4

## EVALUATION OF SPLIT VERSUS SINGLE APPLICATION OF MEADOWFOAM SEED MEAL ON WEED CONTROL

## ABSTRACT

Meadowfoam (*Limnanthes alba* Hartw. *ex* Benth) seed meal (MSM), a by-product of meadowfoam oil extraction, has a secondary metabolite known as the glucosinolate glucolimnanthin (GLN). The breakdown products of GLN are reported to be herbicidal. Two field studies were conducted to compare split and single applications of activated MSM for weed control in lettuce. The activation process was performed by adding 1% by weight of freshly ground meadowfoam seed to MSM. MSM was applied either as a full rate, 2.86 kg $m^{-2}$ on day 0, or as a split rate, 1.43 kg $m^{-2}$ on day 0 followed by 1.43 kg $m^{-2}$ on day 7. In addition to MSM and untreated control plots, urea was used as a nitrogen source in order to account for the fertilizer effect of the seed meal. Urea was applied either as a full rate, 16.8 g $m^{-2}$ on day 0, or as a split rate, 8.4 g $m^{-2}$ on day 0 followed by 8.4 g $m^{-2}$ on day 7. Both MSM applications suppressed weed emergence and growth. MSM applications inhibited spiny sowthistle (*Sonchus asper* (L.) Hill) greater than 95% for emergence and 80% for biomass compared to the untreated control. MSM appeared to be an organic source of nitrogen comparable to urea. GLN and its breakdown products (GBPs) were detected up to 9 days in both MSM applications. The split MSM application provided weed control similar to the single MSM application. For ease of use, a single MSM application as a pre-emergence soil amendment benefits crop yield and weed suppression.

# INTRODUCTION

Meadowfoam (*Limnanthes alba* Hartw. ex Benth.) is a member of the Limnanthaceae family, which is native to southern Oregon and northern California. Meadowfoam is grown as a winter rotation crop in Willamette Valley of Oregon. The oil extracted from meadowfoam seed possesses unique unsaturated long-chain 20:1, 22:1, and 22:2 fatty acids (Knapp and Crane 1995) and an oxidative stability that makes it useful in a wide range of cosmetic and personal care formulations. Meadowfoam seed meal (MSM), a by-product after oil extraction, is 70% of harvested crop yield. At present, MSM has little value.

MSM has 2 to 4% content of a plant secondary metabolite, known as the glucosinolate glucolimnanthin (GLN) (Purdy and Craig 1987). Glucosinolate breakdown products in various plant species have potential uses to suppress seedling emergence (Brown and Morra 1995, 1996; Hansson et al. 2008; Rice et al. 2007). GLN is hydrolyzed by myrosinase enzyme and results in glucosinolate breakdown products (GBPs). These GBPs are 3-methoxybenzyl isothiocyanate (isothiocyanate), 3-methoxyphenylacetonitrile (nitrile), 2-(3-methoxyphenyl)ethanethioamide (thioamide), and 2-(3-methoxyphenyl) acetamide (acetamide) (Stevens et al. 2009). Isothiocyanate and nitrile, prominent GBPs in MSM, have been reported to have herbicidal activity (see Chapter 2; Stevens et al. 2009; Vaughn et al. 1996). The effectiveness of MSM as an herbicidal soil amendment depends on the concentration applied. Low levels of MSM may be a growth stimulant for vegetable crops (Vaughn et al. 2008). At MSM concentrations of greater than 2% by volume or weight, the potential use of MSM as a

bioherbicide was reported for control of the weed species, downy brome (*Bromus tectorum* L.) (Machado 2007; Stevens et al. 2009), and velvetleaf (*Abutilon theophrasti* Medik.) (Vaughn et al. 1996). The herbicidal activity of MSM is substantially increased by augmenting it with ground, enzyme-active meadowfoam seeds, which converts the GLN to GBPs (Stevens et al. 2009).

A greenhouse study confirmed the herbicidal effect of MSM on seedling emergence and growth compared to untreated control (see Chapter 2). A field study suggested that MSM has both fertilizer and bioherbicide effects (see Chapter 3). However, bioherbicide effects were much less than those observed in the greenhouse at the same concentration. The fertilizer effect was measured when MSM was applied at 1.22 kg $m^{-2}$ and at 2.04 kg $m^{-2}$; whereas, there was a bioherbicide effect observed at 2.86 kg $m^{-2}$. The objective of this study was to evaluate the effects of split and full rate applications of MSM on crop yield, weed control, and the longevity of potential bioherbicidal compounds in the soil.

# MATERIALS AND METHODS

## Study Sites and Sample Collection

In the summer of 2012, field experiments were conducted at the Lewis-Brown Horticulture Research Farm, Oregon State University (Oregon, USA; 43º 33´ N, 123º 12´ W). The soil was a Mollisol classified as a Malabon silty clay loam (pH 6, 3.2% OM). Soil and air temperature during the studies were recorded (Figure 4.1A). Plots were hand weeded before starting the experiments. Two field experiments were conducted from July 9 to September 12 and from August 1 to October 5 using a randomized complete block design with four replications. There were five treatments which consisted of two amendment materials (urea and activated MSM) applied either as full or split applications and a non-amended control.

Meadowfoam seed meal[1] was passed through a 1 mm-sieve before use. Activated MSM consisted of 1% ground meadowfoam seed and 99% MSM by weight in order to provide active myrosinase (Stevens et al. 2009). Activated MSM was applied at 2.86 kg $m^{-2}$ which provided a nitrogen rate of at least 168 kg $ha^{-1}$, the recommended rate for leaf lettuce (Hemphill 2010). The application was 2.86 kg $m^{-2}$ on day 0 or 1.43 kg $m^{-2}$ on day 0 followed by 1.43 kg $m^{-2}$ on day 7. To account for the fertilizer effect of the seed meal, urea at 168 kg $ha^{-1}$ was used as a mineral nitrogen source and applied either at 16.8 g $m^{-2}$ on day 0 or at 8.4 g $m^{-2}$ on day 0 followed by 8.4 g $m^{-2}$ on day 7. The plot size was 1.6 $m^2$ with a 1.07 m border between plots and a 1.07 m border around the entire site. A hundred seeds of spiny sowthistle (*Sonchus asper* (L.) Hill) and 100 seeds of Japanese millet

(*Echinochloa frumentacea* Link) were sown in a row in each plot immediately after meal incorporation.

Immediately after MSM application, 24.8 mm of water was applied by overhead irrigation. Four days after the initial incorporation (DAI), 24.8 mm of water was applied prior to the second application of the split MSM and urea treatments (schedule delayed one day in Experiment 2 due to irrigation system maintenance). The irrigation schedule was 8.3 mm of water applied daily from day 7 to day 14 after lettuce transplanting, and then 6.2 mm of water two times a week until 65 DAI.

Leaf lettuce (*Lactuca sativa* L. 'Black Seeded Simpson')[2], was chosen as a short season crop for the studies. This variety has a 45-day maturity and was recommended for transplanting in Oregon (Hemphill 2010). Lettuce seeds were sown in propagation trays[3] in the greenhouse with 25/20 ºC day/night temperature and a 14 hr photoperiod before transplanting. Nine lettuce seedlings (18 d old) were transplanted in the middle row of the plot immediately following the second application of the split MSM and urea applications. Lettuce was transplanted at 15 cm in-row-spacing. One week after transplanting in Experiment 2, four lettuce seedlings (25 d old) were replaced in each plot of the split MSM application because of residual crop injury. On 35 DAI, seven lettuce plants were harvested for aboveground biomass. Seedlings of sown spiny sowthistle and Japanese millet were counted and harvested to quantify aboveground biomass. Naturally occurring weeds within areas of 1.14 $m^2$ were separated by species, counted, and harvested to quantify aboveground biomass on 35 DAI and a second harvest was conducted on 65 DAI. Biomass was dried at 60 ºC for 72 hr, and weighed.

**Glucolimnanthin and Its Breakdown Products**

Soil samples were taken from 0.46 $m^2$ area of each plot using a 2.5 cm diameter soil core. Three soil cores from each plot were sampled at 0-15 cm depth. Soil samples were composited to form one representative sample for each plot and kept in a cool container during transport to the laboratory. Subsamples (approximately 20 g moist soil) were weighed, placed in aluminum cups, dried at 120 ºC for 2 hr, and reweighed to determine soil moisture content. Soil moisture content was measured on 0, 3, 6, 7, 9, 15, and 28 DAI (Figure 4.1B).

The extraction method for extracting glucosinolate and its hydrolysis compounds from the soil was developed by modifying the method of Stevens et al. (2009). Glucolimnanthin (CAS 111810-95-8, S-(β-D-glucopyranosyl)-(Z)-2-(3-methoxyphenyl)-N-(sulfooxy)ethanimidothioic acid potassium salt) was extracted from meadowfoam seed meal. 3-Methoxybenzyl isothiocyanate (isothiocyanate)[4], 3-methoxyphenylacetonitrile (nitrile)[5], and 3-methoxyphenylacetic acid[6] were used as standards for GLN and GBP analyses.

Soil samples (2 g equivalent dry weight) were placed into a 15 ml centrifuge tube. Each tube received 6 ml of 70% methanol. The tube was shaken, sonicated for 10 min, and allowed to stand for 60 min. The mixture was centrifuged for 5 min at 3,000 rpm. The supernatant was centrifuged for 10 min at 13,000 rpm. The methanol concentration in the supernatant was increased to 90% to prevent further enzymatic degradation of GLN. The analyses of GLN and GBPs were performed by using high performance liquid chromatography (HPLC) as described by Stevens et al. (2009). The injection volume was 30 µl. A Waters 2996 photodiode array detector (210 to 500 nm) at 274 nm was used to

calculate peak areas for all compounds. Analyte concentrations were determined from calibration curves constructed for each analyte using the external standard method. The GLN and GBPs were quantified on 0, 3, 6, 9, and 15 DAI. On 0 DAI, the extraction started approximately 5 hr after MSM incorporation.

**Statistical Analyses**

Lettuce biomass, total plant nitrogen and sulfur, and weed emergence and biomass were analyzed using one-way analysis of variance (one-way ANOVA) with means separated using a Tukey's HSD test at a 0.05 level PROC GLM in SAS v. 9.2[7]. GLN and GBP concentrations were tested for treatment, time, and compound composition effects using three-way ANOVA with means separated using a Tukey's HSD test at a 0.05 level in the statistical program R v. 3.0.1 (R Core Team 2013). Weed community data were analyzed using nonmetric multidimensional scaling (NMS) from the multivariate statistical software package PC-ORD v. 6.07[8] (McCune and Mefford 2011).

The main species matrix consisted of weed biomass measured across the 20 sample plots in each experiment. In Experiment 1, there were 31 weed species identified and used in analyses. In Experiment 2, 35 weed species, 34 identified weed species and one group of unidentified Poaceae species, were used in the analyses (see Appendix B.1). The square root transformation of weed biomass was applied to all species to improve distributions along the scale which was appropriate for both high and low biomass values. An ordination of the multidimensional species data was used to investigate patterns of weed species biomass changes in relation to the treatments using nonmetric

multidimensional scaling (NMS) (Kruskal 1964; Mather 1976). The sample plots were ordinated in species space. NMS ordinations used autopilot, slow and thorough, tie penalizing, and Sørensen distance for all runs. Sørensen was selected for its tendency to retain sensitivity in heterogeneous data sets with less weight given to outliers (McCune and Grace 2002).

# RESULTS

The patterns of lettuce growth, plant nutrient, weed emergence and growth, and total concentration of GLN and GBPs differed between the two experiments. The air and soil temperatures and soil moisture between the two experiments were dissimilar and fluctuated (Figure 4.1). Therefore, statistical analyses were performed by experiment.

## Lettuce Growth and Plant Nutrient Availability

The total sulfur across treatments was marginally different in Experiment 1 ($p$ = 0.051) and was different in Experiment 2 ($p$ = 0.02) (Table 4.1). The total plant sulfur in the full MSM application was greater in both experiments. In Experiment 1, mean of total plant nitrogen in plots amended with urea and MSM was 49% greater than those in non-amended plots. In Experiment 2, mean of total plant nitrogen in the urea-amended was not different from the non-amended treatment. Mean of total plant nitrogen between MSM-amended treatments was 41% and 29% greater than non-amended and urea-amended treatments, respectively.

## Weed Emergence and Growth

Total emergence of naturally occurring weeds and spiny sowthistle at 35 DAI differed across treatments (Table 4.2). Within each experiment, the emergence of naturally occurring weeds and spiny sowthistle and Japanese millet was reduced in MSM-amended compared to urea-amended and non-amended treatments. The emergence of naturally occurring weeds, spiny sowthistle, and Japanese millet was not different

between the full and the split MSM application in either experiment. Emergence of Japanese millet was inhibited by MSM-amended treatments in Experiment 1 and by split MSM application in Experiment 2.

The biomass of naturally occurring weeds, spiny sowthistle, and Japanese millet was not different between the full and the split MSM application in either experiment (Table 4.3). At 35 DAI, mean dry biomass of naturally occurring weeds between MSM-amended plots was reduced more than 85% for both experiments compared to non-amended treatment. There was no difference in dry biomass of naturally occurring weeds harvested at 65 DAI. In both experiments, dry weight per plant of Japanese millet was not different across treatments (on average of 0.8 g plant$^{-1}$ for Experiment 1 and 0.7 g plant$^{-1}$ for Experiment 2).

On 35 DAI in Experiment 1, naturally occurring weed emergence was inhibited by 87% in MSM-amended compared to the mean of non-amended and urea-amended treatments (Table 4.2). On 35 DAI in Experiment 2, naturally occurring weed emergence was suppressed by 87% by both MSM amendments compared to the mean of non-amended and the full urea application and by 77% by both MSM amendments over the split urea application. Spiny sowthistle emergence was inhibited more than 95% for Experiment 1 and 100% for Experiment 2 by both MSM applications compared to the non-amended treatment. Japanese millet emergence was suppressed greater than 75% for Experiment 1 by both MSM applications and 83% for Experiment 2 by the split MSM application compared to non-amended treatment. There was no difference of Japanese millet emergence between the full MSM application and the non-amended treatment ($p > 0.05$). Spiny sowthistle was found *in situ* at the Lewis-Brown Horticulture Research

Farm, whereas Japanese millet was not. The analyses for naturally occurring weeds do not include the data of sown spiny sowthistle.

On 65 DAI, there was no difference of naturally occurring weed emergence between the full and the split MSM application. MSM-amended treatments did not inhibit naturally occurring weed emergence in Experiment 1 but did in Experiment 2. In Experiment 2, naturally occurring weed emergence was inhibited greater than 71% by both MSM amendments compared to mean of non-amended and urea-amended treatments.

For ecological community analyses, the differences across amended materials were visible (Figure 4.2). The variations within the same treatment were greater in MSM-amended plots than those in urea-amended or non-amended plots. The two dimensions accounted for 91.7% and 93.7% of the total variation for Experiment 1 and 2, respectively. In both experiments, weed biomass samples from MSM-amended plots had higher coordinate values for the NMS1 than either non-amended or urea-amended plots. Weed composition was more similar between non-amended and urea-amended plots. Based on Pearson's correlation on NMS1 of Experiment 1, common purslane (POROL: *Portulaca oleracea* L.), spiny sowthistle (SONAS), and Japanese millet (ECHCF) were dominant in non-amended and urea-amended treatments (NMS1, $r > |0.5|$, see Appendix B.2). Those species were less likely found in MSM-amended treatments. For Experiment 2, spiny sowthistle (SONAS), field horsetail (EQUAR: *Equisetum arvense* L.), redstem fillaree (EROCI: *Erodium cicutarium* (L.) L'Hér. ex Ait.) and annual bluegrass (POANN: *Poa annua* L.) were predominantly related to non-amended and urea-amended treatments while those species were less likely found in MSM-amended treatments (NMS1, $r > |0.5|$,

see Appendix B.2). Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) was found to a greater extent in MSM-amended plots in Experiment 2 (NMS2, $r$ = -0.66, see Appendix B.2).

**Glucolimnanthin and Its Breakdown Products**

GLN and GBP concentrations were detected up to 9 DAI in MSM-amended treatments in both experiments (Figure 4.3 and 4.4). No detection of GLN or GBP was found in urea-amended and non-amended treatments. The concentrations of GLN and GBPs in the full and the split MSM applications were different in Experiment 1 but were not in Experiment 2 (three-way ANOVA, $p < 0.001$ for Experiment 1; $p = 0.30$ for Experiment 2).

In both experiments, the concentrations of GLN or each GBPs varied across sampling time ($p < 0.001$). Interestingly, the concentration of each compound was not different between the full and split MSM applications. The concentrations of GLN and GBPs of each sampling time were different between the full and split MSM application. There were interactions among application method, sampling time, and compound. In the full MSM application, concentrations of GLN and GBPs decreased more than 50% at 6 and 9 DAI (Figure 4.3A and 4.4A). The concentrations of GLN and GBPs were not different in the full MSM application between 5 hr and 3 DAI nor between 6 and 9 DAI.

In Experiment 1, isothiocyanate was detected only at 5 hr after MSM incorporation with 82% more in the full than in the split MSM applications. Concentrations of nitrile in the full MSM application at 5 hr and 3 DAI were 57% and 86% greater than in the split MSM application, respectively. Nitrile and MPAA were

detected at 5 hr and remained until 6 DAI for nitrile and 9 DAI for MPAA in the full MSM application. After the second application of the split MSM, GLN and MPAA were detectable on 9 DAI (Figure 4.3B).

In Experiment 2, GLN was detectable up to 6 DAI in the full and up to 9 DAI in the split MSM application (Figure 4.4). Nitrile concentrations were greatest at 5 hr in the full and the split MSM applications. Nitrile concentrations remained the same from 3 to 9 DAI in the split MSM application ($p > 0.05$). On 9 DAI, MPAA concentration was 74% greater in the split than in the full MSM application.

# DISCUSSION

Crop growth and phytotoxic effects on weed emergence and growth were not different between the full and the split MSM application. Weed community composition was similar between the full rate and the split MSM applications. GLN and GBP concentrations were different between the full and the split MSM applications on 0 and 3 DAI for Experiment 1 and on 9 DAI for Experiment 2.

There was no difference in lettuce biomass between urea-amended and MSM-amended treatments (Table 4.1). MSM treatment appeared to be an organic source of nitrogen in complex structures of protein, fiber, and GLN (Purdy and Craig 1987). The organic nitrogen supplied from MSM was plant available at a similar or greater level compared to a mineral fertilizer, urea. Similar results were found in conifer seedlings which grew faster in potting medium amended with MSM compared to non-amended medium (Linderman et al. 2007). The conifer seedlings in the MSM treatment had at least 70% greater total nitrogen and lacked nutrient deficiency symptoms, especially phosphorus compared to non-amended control.

In Experiment 2, an irrigation schedule was delayed one day and resulted in high soil moisture of 21.5% on the day of lettuce planting and the second application of the split MSM and urea treatments (Figure 4.1B). Lettuce injury was observed in the split MSM application plots in Experiment 2. The high soil moisture was ideal for the GLN hydrolysis to isothiocyanate by mediation of exogenous myrosinase from freshly ground meadowfoam seed (VanEtten and Tookey 1978). Isothiocyanate has the most herbicidal activity of the glucosinolate breakdown products (see Chapter 2; Brown and Morra 1996;

Morra and Kirkegaard 2002; Vaughn et al. 2006). In Experiment 2, the greater concentrations of GLN or GBPs on 9 DAI in the split compared to those in the full MSM application also indicated the likelihood of phytotoxic compounds (Figure 4.4B). For a split MSM application, schedule adjustment for planting or reapplication of MSM needs to be considered when other factors influencing crop growth and development are involved. The second MSM application should be after crop seedlings are vigorously growing.

The emergence and growth of naturally occurring weeds were inhibited in MSM-amended treatments at 35 DAI (Table 4.2 and 4.3). MSM had high level of suppression on emergence and growth of spiny sowthistle but not on the growth of Japanese millet (Table 4.2 and 4.3). Emerged Japanese millet seedlings from MSM-amended treatments were fewer but larger compared to non-amended and urea-amended treatments because they may have responded to the addition of plant-available nutrients from MSM. Naturally occurring weed communities in the MSM-amended were different from urea amended and non-amended treatments (Figure 4.2). In MSM-amended plots, common purslane in Experiment 1 and field horsetail, redstem filaree, and annual bluegrass in Experiment 2 occurred at lower frequency compared to urea-amended and non-amended plots. However, the herbicidal activity was not enough to suppress fast growing species such as barnyardgrass in late summer.

An interval of 7 d was chosen for the second application of the split MSM because the herbicide effect decreased 6 d after MSM incorporation in a greenhouse study (see Chapter 2). For the split MSM application, greater concentrations of GLN, nitrile, or MPAA were detected on 9 DAI (Figure 4.3 and 4.4). However, the extended

GLN and GBPs in the split MSM application did not provide greater weed suppression compared to the full MSM application. Johnson-Maynard et al. (2005) suggested that the reapplication of seed meal may provide adequate weed control throughout the growing season but also could increase late-season weed biomass due to the increase in plant-available nitrogen.

MSM treatments applied at 2.86 kg $m^{-2}$ and soil incorporated promoted lettuce growth and provided a nitrogen source similar to mineral nitrogen fertilizer and inhibited weed emergence and growth. The early summer application provided better weed control than the late summer application possibly due to less weed pressure in the early summer. Other environmental conditions (e.g. temperature and soil moisture) between the experiments may have led to the difference in GLN and GBP concentrations. The split MSM application provided weed control similar to the full MSM application but needs adjustment in timing and concentration to prevent crop injury. In summary, a single MSM application as a pre-emergence soil amendment is recommended to increase crop yield and weed suppression. As co-occurrence of fertilizer and herbicidal effects of MSM-amended was observed, effects of MSM application on soil quality and soil microbial community still need to be investigated.

## SOURCES OF MATERIALS

[1] Meadowfoam seed meal, Natural Plant Products, Inc., 707 13th St. SE, Suite 275, Salem, OR 97301.

[2] Leaf lettuce 'Black Seeded Simpson', Ferry-Morse Seed Company, 601 Stephen Beale Dr., Fulton, KY 42041.

[3] Propagation tray, Landmark Plastic Corporation, Akron, OH 44306.

[4] 3-Methoxybenzyl isothiocyanate, Oakwood Products, West Columbia, SC 29172.

[5] 3-Methoxyphenylacetonitrile, Sigma-Aldrich Chemicals, St. Louis, MO 63178.

[6] 3-Methoxyphynylacetic acid, TCI America, Portland, OR 97203.

[7] SAS version 9.2, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513.

[8] PC-ORD 6.12, MjM Software Design, PO Box 129, Gleneden Beach, OR 97388.

## LITERATURE CITED

Brown, P. D. and M. J. Morra. 1995. Glucosinolate-containing plant tissues as bioherbicides. J. Agric. Food Chem. 43: 3070-3074.

Brown, P. D. and M. J. Morra. 1996. Hydrolysis products of glucosinolates in *Brassica napus* tissues as inhibitors of seed germination. Plant Soil 181: 307-316.

Hansson, D., M. J. Morra, V. Borek, A. J. Snyder, J. L. Johnson-Maynard, and D. C. Thill. 2008. Ionic thiocyanate ($SCN^-$) production, fate, and phytotoxicity in soil amended with Brassicaceae seed meals. J. Agric. Food Chem. 56: 3912-3917.

Hemphill, D. 2010. Oregon vegetables: lettuce. Available at http://horticulture.oregonstate.edu/content/lettuce-0. Access: May 1, 2011.

Johnson-Maynard, J. M., Morra, L. Dandurand, C. William, and M. J. Butters. 2005. Brassicaceae seed meal application for weed reduction and improved nitrogen management in organic farming systems. Project report, University of Idaho, Moscow, ID.

Knapp, S. J. and J. M. Crane. 1995. Fatty acid diversity of section Inflexae *Limnanthes* (meadowfoam). Ind. Crops Prod. 4: 219-227.

Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika 29: 115-129.

Linderman, R. G., E. A. Davis, and C. J. Masters. 2007. Response of conifer seedlings to potting medium amendment with meadowfoam seed meal. Pages 138-142 in J. Janick and A. Whipkey, eds. Issues in New Crops and New Uses. Alexandria, VA: ASHS Press.

Machado, S. 2007. Allelopathic potential of various plant species on downy brome: implications for weed control in wheat production. Agron. J. 99: 127-132.

Mather, P. M. 1976. Computational methods of multivariate analysis in physical geography. London: John Wiley & Sons. 532 p.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. Gleneden Beach, OR: MjM Software Design. 300 p.

McCune, B. and M. J. Mefford. 2011. PC-ORD. Gleneden Beach, OR: MjM Software Design.

Morra, M. J. and J. A. Kirkegaard. 2002. Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biol. Biochem. 34: 1683-1690.

Purdy R. H. and C. D. Craig. 1987. Meadowfoam: new source of long-chain fatty acids. J. Am. Oil Chem. Soc. 64: 1493-1498.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/.

Rice, A. R., J. L. Johnson-Maynard, D. C. Thill, and M. J. Morra. 2007. Vegetable crop emergence and weed control following amendment with different Brassicaceae seed meals. Renew. Agric. Food Syst. 22: 204-212.

Stevens, J. F., R. L., Reed, S. Alber, L. Pritchett, and S. Machado. 2009. Herbicidal activity of glucosinolate degradation products in fermented meadowfoam (*Limnanthes alba*) seed meal. J. Agric. Food Chem. 57: 1821-1826.

VanEtten, C. H. and H. L. Tookey. 1978. Glucosinolates in cruciferous plants. Page 507-520 in R. F. Keeler, K. R. Van Kampen, and L. F. James, eds. Effects of Poisonous Plants on Livestock. New York: Academic Press.

Vaughn, S. F., M. A. Berhow, and B. Tisserat. 2008. Stimulation of plant growth by (3-methoxyphenyl) acetonitrile applied as a foliar spray *in vivo* or as a medium amendment *in vitro*. HortSci. 43: 372-375.

Vaughn, S. F., R. A. Boydston, and C. A. Mallory-Smith. 1996. Isolation and identification of (3-methoxyphenyl)acetonitrile as a phytotoxin from meadowfoam (*Limnanthes alba*) seedmeal. J. Chem Ecol 22: 1939-1949.

Vaughn, S. F., D. E. Palmquist, S. M. Duval, and M. A. Berhow. 2006. Herbicidal activity of glucosinolate-containing seedmeals. Weed Sci. 54: 743-748.

Table 4.1. Aboveground biomass of seven lettuce plants and chemical analyses per gram of lettuce tissues grown 28 days in the field with meadowfoam meal or urea incorporation.

| Expt | Trt[a] | Lettuce biomass[b] | | Total sulfur | | Total nitrogen | |
|---|---|---|---|---|---|---|---|
| | | ----------g---------- | | ----------g---------- | | ----------g---------- | |
| 1 | NC | 33.6 (2.74) | ns[c] | 0.068 (0.0043) | ns | 1.0 (0.11) | b |
| | UF | 47.2 (4.44) | | 0.092 (0.0101) | | 2.1 (0.18) | a |
| | US | 41.8 (3.06) | | 0.093 (0.0073) | | 2.0 (0.13) | a |
| | MF | 37.2 (3.89) | | 0.112 (0.0092) | | 1.9 (0.15) | a |
| | MS | 36.3 (5.16) | | 0.110 (0.0164) | | 1.8 (0.27) | a |
| 2 | NC | 25.5 (1.92) | ns | 0.061 (0.0046) | ab | 1.0 (0.09) | b |
| | UF | 24.6 (2.55) | | 0.058 (0.0074) | ab | 1.3 (0.03) | ab |
| | US | 25.9 (0.77) | | 0.056 (0.0043) | b | 1.1 (0.05) | ab |
| | MF | 36.9 (5.21) | | 0.099 (0.0159) | a | 1.7 (0.28) | a |
| | MS | 32.4 (2.40) | | 0.091 (0.0072) | ab | 1.7 (0.12) | a |

[a] Treatments: NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

[b] Data are represented as means with SE within parentheses. Different letters within a column indicate significant differences at the 0.05 level using Tukey's HSD within an experiment.

[c] Not significant.

Table 4.2. Emergence of naturally occurring weeds, spiny sowthistle and Japanese millet harvested at different days after initial meadowfoam meal or urea incorporation (DAI).

| Expt | Trt[a] | Emergence[b] naturally occurring weeds | | Spiny sowthistle emergence | Japanese millet emergence |
|---|---|---|---|---|---|
| | | ----------seedlings $m^{-2}$---------- | | ----------% of sown seeds[c]---------- | |
| 1 | | 35 DAI | 65 DAI | 35 DAI | 35 DAI |
| | NC | 150.9 (29.6) a | 4.8 (0.6) ns[d] | 25.8 (2.84) a | 11.8 (2.14) a |
| | UF | 173.9 (22.1) a | 8.8 (2.3) | 24.0 (2.12) a | 12.3 (1.11) a |
| | US | 182.7 (39.3) a | 7.2 (1.1) | 19.5 (3.01) a | 9.5 (1.55) a |
| | MF | 23.2 (5.0) b | 2.0 (0.9) | 1.3 (0.95) b | 3.0 (1.08) b |
| | MS | 13.2 (1.4) b | 2.9 (2.3) | 0 b | 1.3 (0.48) b |
| 2 | | 35 DAI | 65 DAI | 35 DAI | 35 DAI |
| | NC | 308.8 (23.5) a | 229.2 (32.4) a | 29.0 (3.29) a | 10.3 (2.36) ab |
| | UF | 276.3 (38.5) a | 212.1 (13.4) a | 26.8 (3.64) a | 10.8 (1.89) a |
| | US | 166.4 (6.6) b | 160.5 (6.3) a | 20.8 (3.61) a | 8.5 (2.63) ab |
| | MF | 38.6 (7.2) c | 49.8 (10.4) b | 0 b | 3.8 (1.89) bc |
| | MS | 37.9 (3.1) c | 58.1 (5.2) b | 0 b | 1.8 (0.48) c |

[a] Treatments: NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

[b] Data are represented as means with SE within parentheses. Different letters within a column indicate significant differences at the 0.05 level using Tukey's HSD within an experiment.

[c] Average percentage of emerged seeds of the total 100-sown seeds.

[d] Not significant.

Table 4.3. Aboveground biomass of naturally occurring weeds, spiny sowthistle and Japanese millet harvested on different days after initial meadowfoam meal or urea incorporation (DAI).

| Expt | Trt[a] | Dry weight naturally occurring weeds[b] | | Spiny sowthistle | | Japanese millet | |
|---|---|---|---|---|---|---|---|
| | | -----g $m^{-2}$----- | | -----g $m^{-2}$----- | | -----g $plant^{-1}$----- | -----g $plant^{-1}$----- |
| 1 | | 35 DAI | | 65 DAI | | 35 DAI | 35 DAI |
| | NC | 41.0 (10.79) | b | 0.44 (0.079) | ns[c] | 0.13 (0.007) a | 0.48 (0.052) ns |
| | UF | 83.1 (9.45) | a | 2.20 (0.933) | | 0.14 (0.012) a | 0.98 (0.066) |
| | US | 55.9 (13.21) | b | 0.64 (0.238) | | 0.15 (0.017) a | 0.97 (0.077) |
| | MF | 6.3 (0.21) | c | 0.17 (0.087) | | 0.08 (0.046) ab | 0.60 (0.209) |
| | MS | 4.8 (0.32) | c | 1.08 (0.986) | | 0 b | 1.06 (0.715) |
| 2 | | 35 DAI | | 65 DAI | | 35 DAI | 35 DAI |
| | NC | 66.5 (13.74) | a | 29.6 (1.33) | ns | 0.07 (0.011) a | 0.54 (0.195) ns |
| | UF | 60.6 (6.66) | a | 30.7 (6.28) | | 0.07 (0.010) a | 0.36 (0.023) |
| | US | 40.1 (2.96) | b | 27.7 (3.80) | | 0.07 (0.018) a | 0.54 (0.087) |
| | MF | 9.8 (2.02) | c | 20.0 (7.88) | | 0 b | 0.91 (0.174) |
| | MS | 10.3 (0.87) | c | 25.6 (3.93) | | 0 b | 0.97 (0.253) |

[a] Treatments: NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

[b] Data are represented as means with SE within parentheses. Different letters within a column indicate significant differences at the 0.05 level using Tukey's HSD within an experiment.

[c] Not significant.

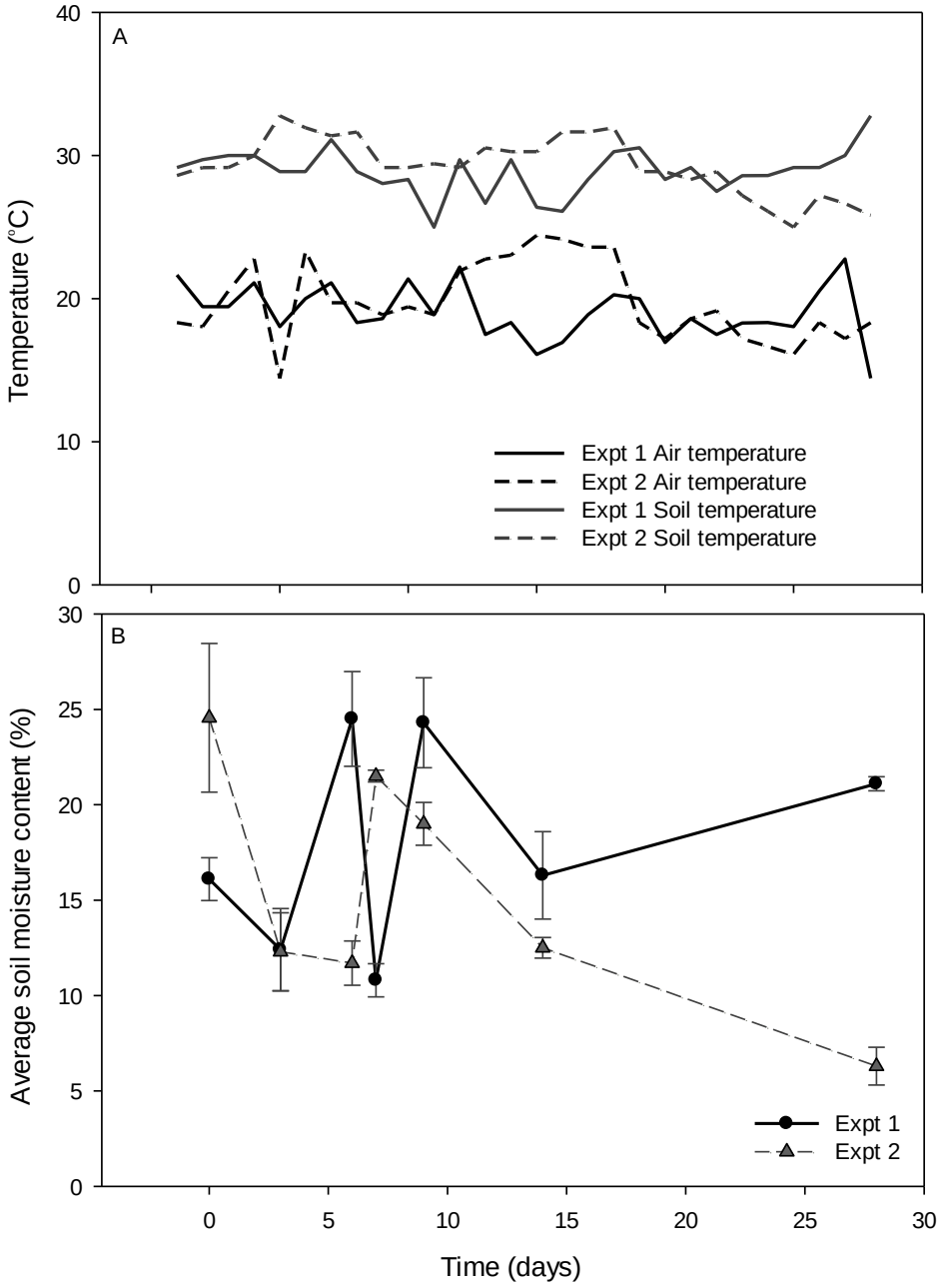



Figure 4.1. Environmental data of average daily air and top 5-cm soil depth temperatures recorded in 2012 at a weather station located in Hyslop Field Lab, Corvallis, Oregon, approximately 13 km from the study site (A) and average soil moisture at top 5-cm soil depth measured from study areas (B).

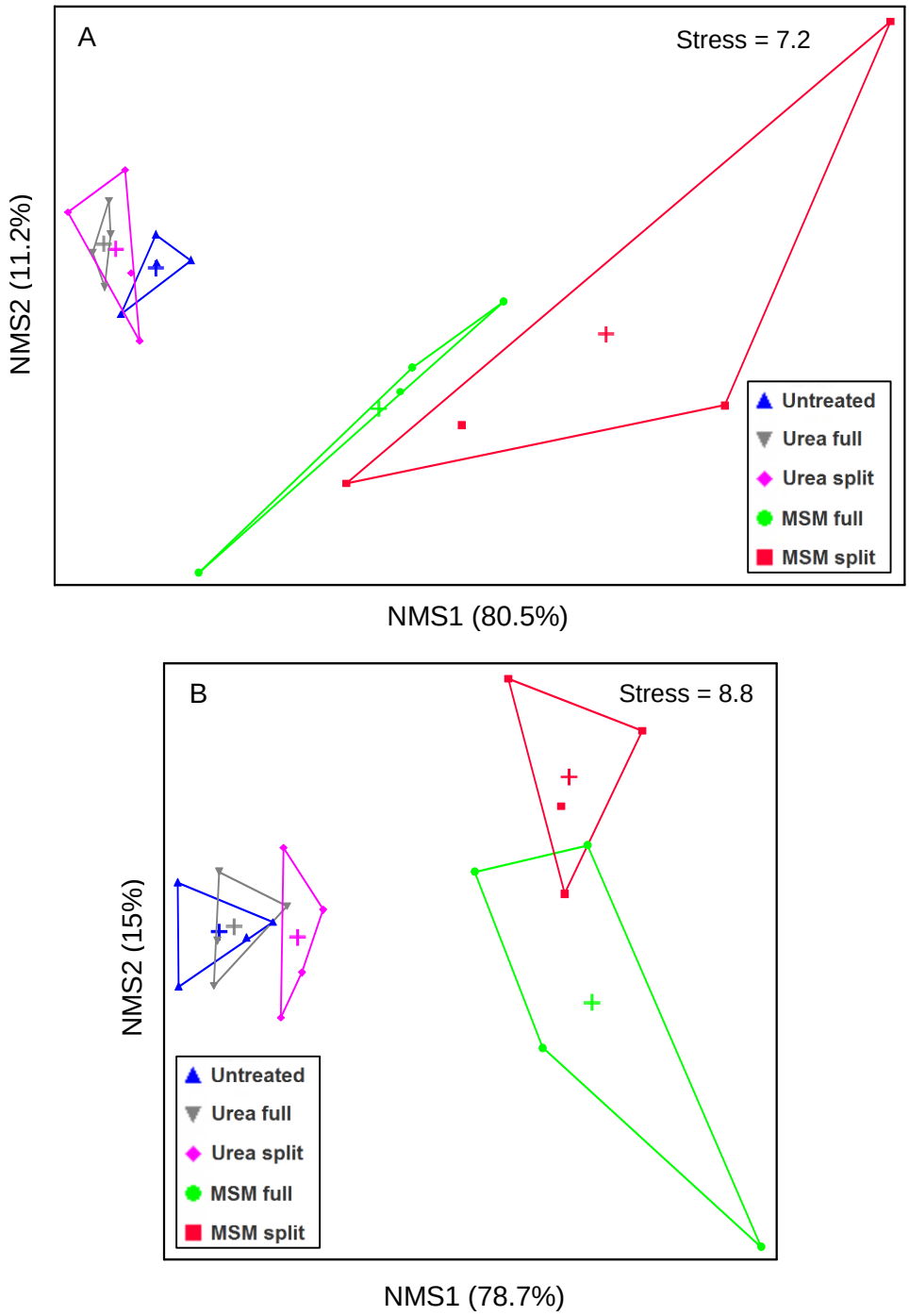



Figure 4.2. Nonmetric multidimensional scaling (NMS) analysis of species composition of weed biomass data harvested 35 days after initial material incorporation in Experiment 1 (A) and Experiment 2 (B). + = centroid of each incorporated treatment. Variance percentage explained by each axis is represented in parentheses. See Appendix B.2 loading variables associated with each NMS axis.

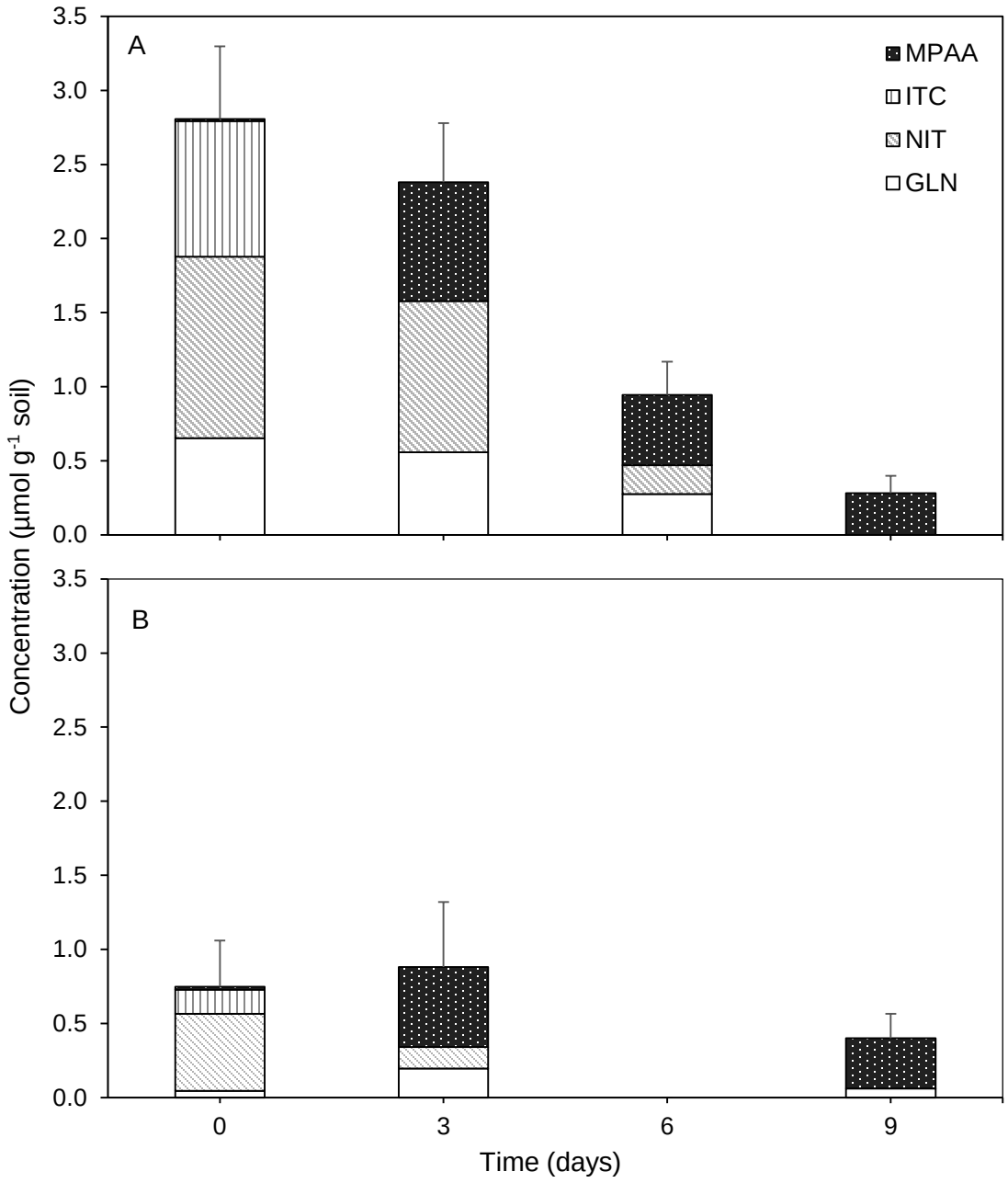



Figure 4.3. Concentrations of glucolimnanthin and its breakdown products in field soil of Experiment 1 amended with full rate of meadowfoam seed meal (A) and split rate of meadowfoam seed meal (B), at 0-15 cm depth. On day 0, the extraction started 5 hr after meal incorporation. MPAA = 3-methoxyphenylacetic acid; ITC = isothiocyanate; NIT = nitrile; GLN = glucolimnanthin.

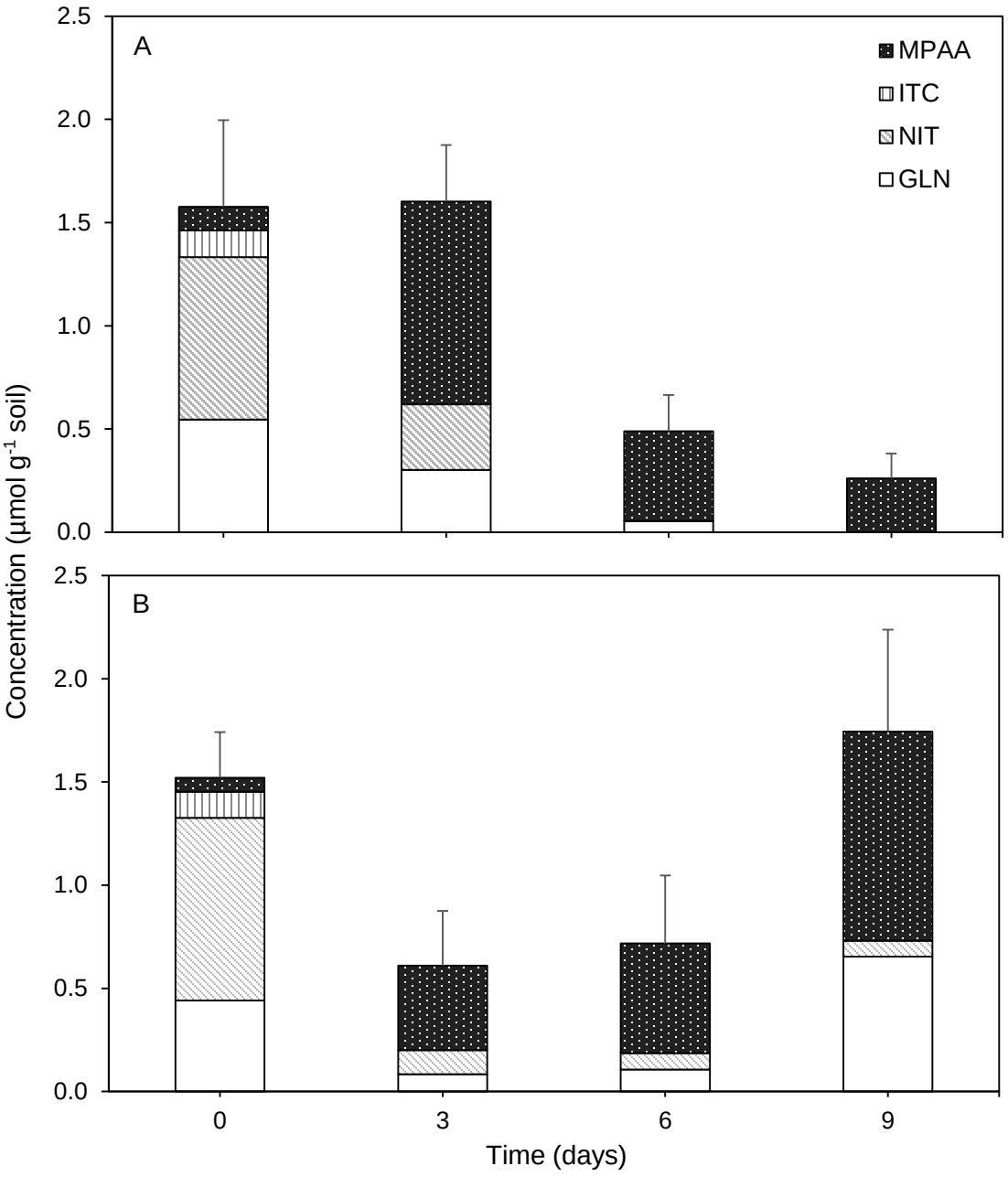



Figure 4.4. Concentrations of glucolimnanthin and its breakdown products in field soil of Experiment 2 amended with full rate of meadowfoam seed meal (A) and split rate of meadowfoam seed meal (B), at 0-15 cm depth. On day 0, the extraction started 5 hr after meal incorporation. MPAA = 3-methoxyphenylacetic acid; ITC = isothiocyanate; NIT = nitrile; GLN = glucolimnanthin.

# CHAPTER 5

## SHORT-TERM EFFECTS OF SOIL AMENDMENT WITH MEADOWFOAM SEED MEAL ON SOIL MICROBIAL COMPOSITIONS AND FUNCTIONS

## ABSTRACT

Meadowfoam (*Limnanthes alba* Hartw. *ex* Benth) seed meal (MSM), a by-product of meadowfoam oil extraction, has a secondary metabolite known as glucosinolate glucolimnanthin. MSM applied as a soil amendment has been reported to have potential herbicidal and fertilizer activities. Studies were conducted to evaluate short-term effects of MSM application on soil microbial communities. MSM was applied either as a full rate, 2.86 kg $m^{-2}$ on day 0, or as a split rate, 1.43 kg $m^{-2}$ on day 0 followed by 1.43 kg $m^{-2}$ on day 7. In addition to MSM and untreated control treatments, urea was used as a nitrogen source in order to account for the fertilizer effect of the seed meal. Urea was applied either as a full rate, 16.8 g $m^{-2}$ on day 0, or as a split rate, 8.4 g $m^{-2}$ on day 0 followed by 8.4 g $m^{-2}$ on day 7. Soil microbial activities were not different between the full and the split rate applications of urea or MSM. Community-level physiological profiling indicated differences in sole-carbon-source utilization between MSM-amended and urea-amended or non-amended treatments. Microbial communities in MSM-amended treatments utilized complex carbon sources from the groups of amine, polymer, and phenolic compounds to a relatively greater degree than microbial communities in urea-amended or non-amended treatments. The carbon and nitrogen inputs from MSM increased the gross metabolic activity of the mixed microbial population. Basal respiration was stimulated and microbes reallocated carbon input to biomass and enzyme production. The reallocation occurred quickly, within 7 to 14 days after MSM

application. Microbial biomass increased at least 80% for carbon and 95% for nitrogen compared to the non-amended control. β-*N*-acetylglucosaminidase and peroxidase activities were highly correlated with microbial biomass nitrogen. This study was conducted over 28 days; therefore, the effects of MSM application on changes of microbial communities need a longer timespan observation.

# INTRODUCTION

Meadowfoam (*Limnanthes alba* Hartw. ex Benth.) is a member of the Limnanthaceae family, which is native to southern Oregon and northern California. Meadowfoam is grown as a winter rotation crop in the Willamette Valley of Oregon. The oil extracted from meadowfoam seed possesses unique unsaturated long-chain 20:1, 22:1, and 22:2 fatty acids (Knapp and Crane 1995) and an oxidative stability that make it useful in a wide range of cosmetic and personal care formulations. About 70% of the seed remains after oil extraction. At present, this by-product, known as meadowfoam seed meal (MSM), has little value.

MSM applied as a soil amendment has potential herbicidal and fertilizer activities (see Chapter 3 and 4). MSM has 2 to 4% content of a plant secondary metabolite known as the glucosinolate glucolimnanthin (GLN). GLN breakdown products have been reported to have potential herbicidal activity (Stevens et al. 2009; Vaughn et al. 1996).

Changes in soil environment can be measured through changes in soil microorganisms because they are the primary consumers at the soil trophic level. Soil microorganisms, mainly bacteria and fungi, play important roles in nutrient availability for plants (Wardle and Ghani 1995). The variability in a microbial community can be used to indicate a change in soil quality (Breure 2005). However, there are no data on soil microbial activity changes related to MSM application.

Respiration, or $CO_2$ evolution, is a traditional index that measures the gross metabolic activity of mixed microbial populations (Stotzky 1997). Microbial biomass pool sizes are important tools as indicators of soil quality and understanding of nutrient

dynamics (Insam 2001). Jenkinson (1976) proposed an indirect method of microbial biomass determination by chloroform fumigation. In the fumigation process, chloroform kills and lyses microbial cells. The microbe's cytoplasm is released into the soil which is extracted as organic carbon (C) and nitrogen (N) using appropriate extraction methods (Brookes et al. 1985; Vance et al. 1987).

Community-level physiological profiling (CLPP) using the Biolog assay can provide an indication of the functional activity and diversity of the microbial community (Garland and Mills 1991). Color development in Biolog plates is caused by the reduction of tetrazolium violet as indicator of respiration of sole carbon sources. The Biolog technique is simple and rapid and is based on the utilization of organic carbon substrates that are essential for microbial growth. However, it works only with culturable microorganisms and not for fungi due to their slow growth (Nannipieri et al. 2002).

Extracellular enzymes catalyze the initial step of decomposition and nutrient mineralization (Sinsabaugh et al. 2005). Extracellular enzymes are necessary to breakdown the macromolecules, such as cellulose, hemicelluloses or lignin, whereas intracellular enzymes are for the breakdown of smaller molecules such as sugars or amino acids (Insam 2001). Extracellular enzyme activities (EEA) respond quickly to the changes in soil management practices (Doran et al. 1996) and are sensitive indicators of ecological changes (Wallenstein and Weintraub 2008). The enzyme assays are simple, accurate, sensitive, and relatively rapid (Nannipieri et al. 2002) but may not reflect the actual microbial activity (Insam 2001).

The goal of this study was to investigate short term effects of MSM application on soil microbial community. To have a broad picture of microbial composition and

function, we focused on flux measurements of C via basal respiration, soil microbial pools (microbial biomass C and N), and soil enzyme activity (31 sole-carbon sources and five common enzymes). Some common enzymes found in environmental samples (German et al. 2011) were chosen for our studies including three hydrolytic, β-glucosidase, β-*N*-acetylglucosaminidase, acid phosphatase and two oxidative enzymes, phenol oxidase, and peroxidase. Hydrolytic enzymes are involved in carbohydrate and protein degradation and oxidative enzymes are linked with lignin degradation (Sinsabaugh et al. 2002).

# MATERIALS AND METHODS

## Study Sites and Sample Collection

Field studies were conducted on the Lewis-Brown Horticulture Research Farm, Oregon State University (Oregon, USA; 43º 33´ N, 123º 12´ W). The soil was a Mollisol classified as a Malabon silty clay loam (pH 6, 3.2% OM). Average soil and air temperature during the studies were recorded (Figure 5.1A). Plots were cleared of weeds before starting the experiments. Two field experiments were conducted from July 9 to September 12, 2012, for the first experiment and from August 1 to October 5, 2012, for the second experiment using a randomized complete block design with four replications. There were five treatments, two amendment materials (urea and activated MSM) with two application methods (either full or split rate application) and one control treatment (non-amended treatment).

Meadowfoam seed meal[1] (organic C, 407 g $kg^{-1}$; total N, 44.8 g $kg^{-1}$) was passed through a 1 mm-sieve before use. Activated MSM was prepared by adding 1% by weight of freshly ground meadowfoam seeds to MSM in order to provide active myrosinase essential for hydrolysis of GLN (Stevens et al. 2009). Activated MSM was applied at 2.86 kg $m^{-2}$ which provided a nitrogen rate of at least 168 kg $ha^{-1}$, the recommended rate for leaf lettuce (*Lactuca sativa* L. 'Black Seeded Simpson')[2] (Hemphill 2010). The application was 2.86 kg $m^{-2}$ on day 0 or 1.43 kg $m^{-2}$ on day 0 followed by 1.43 kg $m^{-2}$ on day 7. To account for the fertilizer effect of the seed meal, urea at 168 kg $ha^{-1}$ was used as a mineral nitrogen source and applied either at 16.8 g $m^{-2}$ on day 0 or at 8.4 g $m^{-2}$ on day

0 followed by 8.4 g $m^{-2}$ on day 7. The plot size was 1.6 $m^2$ with a 1.07 m border between plots and a 1.07 m border around the entire site.

Immediately, after MSM application, 24.8 mm of water was applied by overhead irrigation. Overhead irrigation was started on day 4 for Experiment 1 and on day 5 for Experiment 2 with 24.8 mm of water. The irrigation schedule was 8.3 mm of water per day from day 7 to day 14, and then 6.2 mm of water, 2 times a week until day 28.

Plot areas were divided for plant evaluation (1.1 $m^2$) (see Chapter 4), and for soil sampling (0.5 $m^2$). Soil samples were taken within the 0.46 $m^2$ in each plot to 5-cm depth. The samples were collected on 0, 7, 14, and 28 d. Three soil cores were sampled in each plot and composited. The moist soil was passed through a 3-mm sieve. Subsamples (approximately 20 g moist soil) were weighed, placed in aluminum cups, dried at 120 ºC for 2 hr, and reweighed in order to determine the soil moisture content. The soil moisture content was measured on 0, 7, 14, and 28 d (Figure 5.1B).

**Basal Respiration**

The fresh soil was incubated in the dark for 48 hr at 25 ºC before monitoring the total $CO_2$ basal respiration using an Isotopic $CO_2$ Analyser[3]. The basal respiration was reported as μg of $^{12}C$ and $^{13}C$ carbon dioxide per gram of dry soil per hour (μg $CO_2$-C $g^{-1}$ $hr^{-1}$).

**Microbial Biomass**

Moist soil (10 g equivalent dry weight) was placed in a 30 ml glass beaker and fumigated with $CHCl_3$. The fumigation was performed in a desiccator containing a

beaker with 50 ml of $CHCl_3$ and anti-bumping granules. The desiccator was evacuated until the $CHCl_3$ boiled vigorously. The desiccator was left in the dark at 25 ºC for 24 hr. The soil samples were removed from the desiccator and the $CHCl_3$ vapor in soil samples was evaporated under a hood for 2 hr. Organic C and N were extracted from fumigated and nonfumigated soil (10 g dry weight) using 50 mL of 0.05 M $K_2SO_4$ solution. The mixture was shaken for 1 hr and filtered using Whatman No.1 filter paper. The filtered solution was collected in a 40-ml vial. The extractable organic C and N were quantified using combustion /chromatography analysis with a total organic carbon[4] and nitrogen analyzer[5]. Potassium hydrogen phthalate ($C_8H_5KO_4$) and ammonium sulfate ($(NH_4)_2SO_4$) were used as external standards for the measurement of organic C and N, respectively. Microbial biomass carbon (MBC) and nitrogen (MBN) were measured as the difference in soil dissolved organic C and N between chloroform ($CHCl_3$) fumigated and nonfumigated soil samples (Brookes et al. 1985; Jenkinson and Powlson 1976). Final MBC and MBN values were calculated using the extraction efficiency coefficient of 0.45 and 0.56 for MBC and MBN, respectively (Brookes et al. 1985; Vance et al. 1987).

**Community-level Physiological Profiling**

The CLPP assay was performed immediately after soil collection using Biolog EcoPlates™ [6]. The plate contains 31 of the most useful carbon sources for soil community analysis and water as a control (see Appendix C.1). The protocol for CLPP was developed by modifying the methods of Singler (2004). Soil sample (5 g equivalent dry weight) was added to a 50-ml centrifuge tube containing 45 ml of sterile 10 mM phosphate buffer (PBS) (pH 7). The tube was shaken at 25 ºC, 200 rpm for 30 min. The

soil solution was diluted to generate approximately $10^5$ colony-forming units (cfu) per 100 µl of soil solution. A serial dilution was performed in a sterile 15-ml centrifuge tube to obtain approximately 12 ml of each inoculum. Inoculum (100 µl) was transferred into each well of the plate. The first measurement of microbial activity was conducted immediately after inoculum transfer using a microtiter plate reader[7] at 590 nm. The plate was incubated at 25 ºC and the activity in the plates was measured on 24, 48, 72, and 144 hr after the first measurement. The color development at 72 hr was chosen for analyses as was recommended by Haack et al. (1995).

**Extracellular Enzyme Activity**

Soil samples frozen at 4 ºC were used in this study. The activity of 5 extracellular enzymes, β-glucosidase (BG), β-*N*-acetylglucosaminidase (NAG), acid phosphatase (PHOS), phenol oxidase (PHENOX), and peroxidase (PEROX) were measured using assay techniques modified from Stursova et al. (2006) and Zeglin et al. (2007). Assays were conducted at 25 ºC, in 50 mM, pH 5 sodium acetate (NaOAc) buffer. Soil suspensions were prepared by adding 1 g dry soil to 100 ml NaOAc buffer and shaking before dispensing into 96-well microplates.

Hydrolytic enzyme assays were performed in black 96-well microplates[8] for fluorescent measurement. The fluorescent substrates for testing the activities of BG, NAG, and PHOS were 4-methylumbelliferone (MUB)-β-D-glucoside[9], 4-MUB-*N*-acetyl-β-D-glucoside[10], and 4-MUB-phosphate[11], respectively. There were 16 replicate control wells on each plate: negative substrate controls (8 wells) and negative sample controls (6 wells sample$^{-1}$). In addition, there were reference standards (8 wells), quench controls (3

wells sample$^{-1}$), and assay wells (6 wells sample$^{-1}$). The negative substrate control wells received 200 µl buffer and 50 µl of substrate. The negative sample control contained 50 µl buffer and 200 µl of sample. Reference standard wells received 200 µl buffer and 50 µl of 10 µM MUB. Quench controls contained 200 µl sample suspension and 50 µl of 10 µl MUB. Assay wells received 200 µl sample suspension and 50 µl substrate. The assays were incubated at 25 ºC for 3 hr and the reactions were terminated by adding 10 µl of 0.5 N NaOH to each well. Fluorescence was measured using a Spectramax Gemini XS Spectrophotometer[12] with excitation at 365 nm and emission at 445 nm.

Oxidative enzyme assays were conducted in clear 96-well microplates[13]. The substrates were L-3,4-dihydroxyphenylalanine[14] for PHENOX and L-3,4-dihydroxyphenylalanine with the additional of $H_2O_2$ for PEROX. Negative substrate controls (8 wells), negative sample controls (4 wells sample$^{-1}$), and assay wells (7 wells sample$^{-1}$) were included. The negative substrate and sample controls were prepared as previously described. For the PEROX assay, each well including the negative control and blank received 10 µl of 0.3% $H_2O_2$. The assay plates were incubated at 25 ºC for 26 hr. Enzyme activity was measured spectrophotometrically at 450 nm absorbance in a microtiter plate reader[7]. All enzyme activities were reported as nmole of substrate converted per gram of dry soil per hour (nmol g$^{-1}$ hr$^{-1}$).

**Statistical Analyses**

Basal respiration, microbial biomass, and EEA data were normalized by natural log transformation before performing statistical analyses in order to meet the assumption of equal variances. Basal respiration, microbial biomass, and EEA data were analyzed

using a two-way analysis of variance (two-way ANOVA) with means separated using Tukey's HSD test at a 0.05 level in the statistical program R v. 3.0.1 (R Core Team 2013). Correlation analyses were performed on microbial biomass and enzyme activity data using a Pearson correlation test PROC CORR in SAS v. 9.2.[15]

The patterns of CLPP in Biolog EcoPlates™ were expressed as a difference in color development between the response well and the control well. The response data were normalized by dividing with average metabolic response (AMR). The normalized data were used to classify samples among and within soil microbial communities using principal component analysis (PCA) (Garland and Mill 1991). AMR was calculated from the mean difference among response values of the 31 carbon source containing wells (Equation 1),

$$\mathrm{AMR} = \frac{\sum(O.D.well - O.D.control\ well)}{31} \qquad [1]$$

where (O.D. well – O.D. control well) is the optical density of each carbon source containing well minus the optical density from the negative control well.

PCA analyses were performed using the multivariate statistical software package PC-ORD v. 6.12[16] (McCune and Mefford 2011). Correlations are reported using the Pearson's correlation statistic with Euclidean distance.

# RESULTS

The effects of soil amendment with MSM on microbial activity were studied. We investigated the soil microbial community by the composition of microbial fluxes and pools, and soil microbial enzyme activities. The patterns of total $CO_2$ basal respiration, microbial biomass, sole-carbon-source utilization, and enzyme activity in the soil microbial community varied between the two experiments. The air and soil temperatures and soil moisture between the two experiments were dissimilar and fluctuated (Figure 5.1). Therefore, statistical analyses were performed by experiment.

## Basal Respiration

At the same sampling time, no difference of total $CO_2$ basal respiration was detected across non-amended and urea-amended treatments for either experiment (see Appendix C.2). The decreases in basal respiration from the 0 to 28 d were observed in the full MSM and urea applications in both experiments and in the split MSM and urea applications in Experiment 2 (see Appendix C.2). The basal respiration over 28 d was greater in MSM-amended than non-amended treatments ($p < 0.05$) (Figure 5.2). No difference in basal respiration rate between the full and the split MSM application was detected in either experiment, except on 0 d of Experiment 1. On 0 d of Experiment 1, basal respiration was greater in the full MSM application (Figure 5.2A). A new input of MSM in the split application on 7 d did not increase basal respiration compared to the full MSM application ($p > 0.05$). The change of the basal respiration decreased steeply in MSM-amended over 28 d but slightly decreased in non-amended treatments.

**Microbial Biomass**

MBC and MBN varied across non-amended and MSM-amended treatments and sampling times (Figure 5.3 and 5.4). On 0 and 28 d in Experiment 1, MBC concentrations among non-amended and MSM-amended treatments were not different, while MBN concentrations in MSM-amended were greater than non-amended treatments (Figure 5.3). On 0 d in Experiment 2, MBC and MBN concentrations were not different among MSM-amended and non-amended treatments (Figure 5.4). On 28 d in Experiment 2, MBC and MBN concentrations in MSM-amended were greater than non-amended treatments.

On 7 and 14 d in both experiments, microbial biomass concentrations in MSM-amended treatments were at least 80% greater for MBC and 95% greater for MBN than non-amended treatments. In Experiment 2, the MBN of the split MSM was 86% greater than the full MSM application on 28 d (Figure 5.4B).

**Community-level Physiological Profiling**

CLPP is another enzyme assay with more carbon sources. The AMR was calculated over the color development of the five soil-amended treatments at four different sampling times (see Appendix C.3 and C.4). Color development in non-amended and urea-amended samples did not reach maximum within 144 hr incubation, whereas MSM-amended samples reached maximum at 72 hr incubation on 7 and 14 d. Chemical guilds included amines, amino acids, carbohydrates, carboxylic acids, polymers, and phenolic compounds (see Appendix C.1).

Soil collected from different treatments had distinctive patterns of sole-carbon-source utilization based on PCA of color response data (Figure 5.5). In both experiments,

differences among amended materials were greater than differences within methods of application, especially in MSM-amended plots. Soil samples from the majority of MSM-amended plots had lower coordinate values for the first principal component (PC) than non-amended or urea-amended plots.

In Experiment 1, PC1 and PC2 explained 20.8% and 10.5% of the variance, respectively (Figure 5.5A). Analysis of PC1 indicated that soil microbial communities in MSM-amended plots utilized amine (phenylethylamine), polymer (α-cyclodextrin), phenolic compounds (2-hydroxy benzoic acid), and carbohydrate (*i*-erythritol) to a relatively greater degree than soil microbial communities from non-amended or urea-amended plots (see Appendix C.5). Eight samples from MSM-amended treatments (Figure 5A; PC1 score > -2) collected on 0 d (four samples each for the full and split applications) fell in the non-amended and urea-amended groupings. The plots with higher PC2 scores had the greater correlation with D-xylose and L-serine carbon sources, while the plots with lower PC2 scores had greater correlation with L-phenylalanine and 4-hydroxy benzoic acid.

In Experiment 2, PC1 and PC2 explained 30.3% and 9% of the variance, respectively (Figure 5.5B). Analysis of PC1 indicated that soil microbial communities in MSM-amended plots utilized amine (phenylethylamine), polymers (α-cyclodextrin and glycogen), and phenolic compounds (2-hydroxy benzoic acid) to a relatively greater degree, than soil microbial communities from non-amended or urea-amended plots (see Appendix C.5). Twelve samples from MSM-amended treatments (Figure 5.5B; PC1 score > -2.5) were collected on 0 and 28 d (four samples each for the full and split applications collected on 0 d, and two samples each for the full and split applications

collected on 28 d) fell in the non-amended and urea-amended groupings. The higher PC scores of sampling plot on the PC2 represented the greater correlation with a carbohydrate (α-D-lactose), whereas the lower PC scores correlated with amino acids (L-phenylalanine).

Carbon sources such as carbohydrates (*N*-acetyl-D-glucosamine, D-cellobiose, D-galactonic acid γ-lactone, and D-mannitol), carboxylic acid (D-galacturonic acid), and amino acids (L-asparagine) were utilized to a relatively greater degree by soil microbial communities in non-amended and urea-amended than MSM-amended plots in either experiment.

**Extracellular Enzyme Activity**

Extracellular enzymes produced from soil microorganisms varied across treatments and sampling times (Figure 5.6). In both experiments, MSM-amended and urea-amended treatments had no effect on BG activity compared to non-amended treatment (Figure 5.6A and 5.6B). In Experiment 1, BG activity in the full MSM application was 55% greater than in the full urea application on 7 d and BG activity in the split MSM application was 53% greater than in the full urea application on 14 d (Figure 5.6A). In Experiment 2, BG activity was not different across treatments (Figure 5.6B).

In Experiment 1, NAG activity was greatest with the full MSM application, followed by the split MSM and the split urea treatments (Figure 5.6C). NAG activity in MSM-amended treatments gradually increased until 28 d. On 28 d, average NAG activity in MSM-amended treatments was 58% greater than the average of urea-amended and non-amended treatments. In Experiment 2, NAG activity with the split MSM and the

non-amended was greater than with the urea-amended treatments (Figure 5.6D). On 28 d, NAG activity of the split MSM and the non-amended was 80% greater than the split urea application.

At the same sampling time, PHOS activity was not different across treatments in either experiment (Figure 5.6E and 5.6F). In Experiment 1, PHOS activity fluctuated slightly over 28 d (Figure 5.6E). In Experiment 2, all treatments had the same patterns of PHOS activity with maximum on 7 d and gradually decreasing until 28 d (Figure 5.6F).

Among oxidative enzymes, the activity changes across treatments and times were more obvious for PEROX than for PHENOX (Figure 5.7). At the same sampling time, PHENOX activity was not different across treatments in either experiment (Figure 5.7A and 5.7B). In Experiment 1, PEROX activity was greater in urea-amended and non-amended than MSM-amended treatments (Figure 5.7C). On 0 d, average PEROX activities among urea-amended and non-amended treatments were 68% greater than the split MSM application. On 7 d, average PEROX activities among urea-amended and non-amended were 54% and 82% greater than the full and the split MSM applications, respectively. Average PEROX activities among urea-amended and non-amended on 14 and 28 d were 76% and 77% greater than average PEROX activities of MSM-amended treatments, respectively. In Experiment 2, PEROX activity was not different across amended and non-amended treatments on 0, 7, and 28 d (Figure 5.7D). On 14 d, PEROX activity in urea-amended was 62% greater than the split MSM application.

**Microbial Biomass Content and Enzymatic Activity**

Microbial biomass and enzyme activity from non-amended and MSM-amended treatments were correlated (Table 5.1). Significant relationships were found between MBC, MBN, BG, NAG, PHOS, PHENOX, and PEROX. Microbial biomass C and N were correlated ($r = 0.80$, $p < 0.001$ for Experiment 1; $r = 0.93$, $p < 0.001$ for Experiment 2). Microbial biomass C and N were positively correlated with hydrolytic enzyme activities (BG, NAG, and PHOS) and negatively correlated with PEROX activity in Experiment 1 ($p < 0.001$) but not in Experiment 2 ($p > 0.05$). Correlations between NAG and PHOS were high in both experiments ($r = 0.66$, $p < 0.001$ for Experiment 1; $r = 0.55$, $p < 0.001$ for Experiment 2). Correlations between PHENOX and PEROX were found in both experiments ($r = 0.42$, $p < 0.01$ for Experiment 1; $r = 0.64$, $p < 0.001$ for Experiment 2). In Experiment 1, BG had positive correlation with NAG, and PHOS activities and negative correlation with PEROX activity ($p < 0.001$). In Experiment 2, BG had relationship with PHOS ($r = 0.53$, $p < 0.001$).

# DISCUSSION

Basal respiration reflects the availability of C for microbial growth and maintenance and is a measure of the basic turnover rate in soil (Insam et al. 1991). Greater basal respiration rate in MSM-amended compared to non-amended treatments was due to organic inputs from MSM. On 0 d, the greater concentration of the full MSM application resulted in greater basal respiration compared to the split rate application. The decrease in basal respiration in MSM-amended treatments over 28 d reflected the decomposition of the MSM. Reduced respiration after soil amendment with MSM was similar to the results of Zaccardelli et al. (2013) using Ethiopian mustard (*Brassica carinata* (A.) Braun) and sunflower (*Helianthus annuus* L.) meals as soil amendment materials. The decrease in basal respiration was observed in both the full MSM and the full urea applications (see Appendix C.2). Reduced respiration after adding mineral N sources had been suggested to reflect microbial reallocation of C to biomass or enzyme production (Schimel and Weintraub 2003).

Microbial biomass reflects nutrient pool, nutrient dynamic, and is an early indicator of soil quality (Insam 2001). MBC and MBN in the control were the least of any treatment and remained unchanged or slightly decreased over 28 d probably due to inadequate nutrient supply in the system. MSM-amended treatments increased MBC and MBN on 7 d or 14 d when basal respiration steeply decreased. However, microbial biomass subsequently declined after 14 d indicating short-term effects of MSM on microbial biomass pools of C and N.

In agricultural soils, metabolic quotient ($qCO_2$) calculated by the ratio of microbial respiration to MBC, reflects nutrient turnover and utilization of C (Insam et al. 1991). Average $qCO_2$ of MSM-amended treatments on 7 and 14 d was 0.22 for Experiment 1 and 0.26 for Experiment 2, and on 28 d, average $qCO_2$ decreased to 0.12 in both experiments. Average $qCO_2$ between 7 and 28 d of non-amended control was 0.05 in either experiment. The greater $qCO_2$ from MSM-amended than from non-amended treatments indicated more evolved $CO_2$ per unit biomass possibly due to the readily available source of C from MSM for microbial growth, maintenance, and respiration. The $qCO_2$ can indicate microbial efficiency; however, we may not reach the steady-state condition of $qCO_2$ on 28 d.

Sole-carbon-source utilization and specific enzyme activities provided more information of soil microbial community and function using the Biolog assay (Garland and Mills 1991). CLPP has been introduced as a classification tool for microbial communities on the basis of heterotrophic metabolism. On 7 d, the dominant carbon sources utilized by microbial communities in MSM-amended soils shifted from those in MSM-amended on 0 d, urea-amended, and non-amended treatments. On 7 and 14 d for both experiments, microbial communities in MSM-amended treatments utilized carbon sources with complex structures of cyclic and aromatic ring in groups of amine, polymer, and phenolic compound (phenylethylamine, α-cyclodextrin, and 2-hydroxy benzoic acid). In contrast, major carbon source utilization in MSM-amended on 0 d (and in some MSM-amended plots on 28 d of Experiment 2), urea-amended, and non-amended treatments utilized simple degradable structures in groups of carbohydrates, carboxylic acids, and amino acids. Enzyme production is N and energy intensive; microbes theoretically

produce enzymes at the expense of growth and metabolism when available nutrients are scarce (Koch 1985). Insufficient supplies of easily degradable carbon sources as well as more diversity of carbon sources from MSM applications may be driving factors of shifting carbon source utilization from the simple substrates to the complex substrates. The different patterns of the major carbon-source utilization in MSM-amended treatments between the studies may possibly be due to environmental and biological conditions.

Organic residue-amended materials stimulated microbial respiration and enzyme activities compared to untreated soil (Zaccardelli et al. 2013). The addition of complex substrates increased the production of extracellular enzymes to utilize new sources for assimilation (Allison and Vitousek 2005). Significantly higher NAG and lower PEROX activities resulted from MSM applications. NAG and PEROX activities were negatively correlated. Other enzymes did not respond to MSM applications possibly due to insufficient resources to stimulate microbial enzyme production. Allison and Vitousek (2005) reported an increase in enzyme activity when target nutrients were from complex sources (e.g. cellulose, collagen, or cellulose phosphate) and there was availability of simple sources of C and N.

NAG is involved in the degradation of chitin and other β-1,4-linked glucosamine polymers, analogous to the role of BG in cellulose degradation (Sinsabaugh et al. 2005). In Experiment 1, NAG activity increased until 28 d. On 28 d, NAG activity in MSM-amended was greater than urea treatments. The greater production of NAG possibly was due to the complex N supplied from MSM. MSM provides 25% protein, 22% fiber, and 4% glucolimnanthin (Purdy and Craig 1987) which can be utilized as C and N sources.

The NAG activity was related to CLPP results in that microbial communities in MSM treatments utilized complex carbon sources. N content in organic materials indicate litter quality and decomposition rate (Sinsabaugh et al. 1993). NAG activity tends to be high during the early stages of decomposition of fast-decomposing litter (Sinsabaugh et al. 2002). Organic materials with C to N ratio below 20 lead to N mineralization into the system (Myrold 2005). MSM has a C to N ratio of 9 which possibly allowed net ammonium production as an available N source for plant uptake.

PEROX enzymes use $H_2O_2$ as an electron acceptor (Hofrichter 2002). In soil, fungi produce various PEROX enzymes to degrade lignin (Sinsabaugh 2005). Oxidative enzyme activity has been related to the concentration of soluble phenolic compounds, the lignin content of amendment materials, soil pH, and nitrogen availability and has opposite responses to hydrolytic enzyme activities (Sinsabaugh 2010). PEROX activity was greater in urea-amended and non-amended compared with MSM-amended treatments. It was possible that the soil pH increased with urea and an insufficient N supply in non-amended soils caused the greater PEROX activities. Some studies reported that PEROX decreased with mineral N amendment (e.g. Sinsabaugh et al. 2005) but did not in other studies (e.g. Zeglin et al. 2007). However, the PEROX enzymes are less stable in the soil condition compared to other hydrolase enzymes (Sinsabaugh 2010).

Correlations between enzyme activity and microbial biomass varied across experiments. More correlations occurred among enzyme activity or between enzyme activity and microbial biomass in Experiment 1 than in Experiment 2. MBC and MBN had positive correlation with enzymes involved in the acquisition of C and N from organic sources in Experiment 1 but not in Experiment 2. In Experiment 1, high

correlations were observed among hydrolytic enzymes. Similar results were found in a study of Sinsabaugh et al. (1993). Activity of different enzymes varied in different environments due to the diversity of soil organic matter, the diversity of soil community, and the diversity of the physical and chemical properties (Wallenstein and Weintraub 2008).

MSM-amended treatments stimulated basal respiration, MBC and MBN, utilization of complex carbon sources, and some enzyme activities related to the acquisition of nitrogen. Microbial biomass C and N increased with MSM-amended over non-amended treatments. Soil microbial activities influenced by MSM-amended treatments were greater in Experiment 1 than in Experiment 2. MSM incorporation induced changes on the structure and function of the soil microbial communities. The changes were mostly related to microbial substrate availability derived from soil amendment with fresh organic materials. In the short-term, the C cycle in MSM amended soil was balanced due to less C loss (respiration) than C assimilated (microbial biomass). Degradation occurred within 7 to 14 d with increasing MBC and MBN, and high acquisition of complex carbon-source utilization. Greater NAG activity in MSM application was related to N acquisition. The benefits of MSM application on soil quality due to basic changes of microbial communities needs further studies to detect the effects of MSM over a longer timespan and explore C and N mineralization.

## SOURCES OF MATERIALS

[1] Meadowfoam seed meal, Natural Plant Products, Inc., 707 13th St. SE, Suite 275, Salem, OR 97301.

[2] Leaf lettuce 'Black Seeded Simpson', Ferry-Morse Seed Company, 601 Stephen Beale Dr., Fulton, KY 42041.

[3] Isotopic $CO_2$ Analyser, Picarro G2101, Picarro Inc., 3105 Patrick Henry Dr., Santa Clara, CA 95054.

[4] Total organic carbon analyzer, TOC-VCSH, Shimadzu Scientific Instruments, Inc., 7102 Riverwood Dr., Columbia, MD 21046.

[5] Total nitrogen analyzer, TNM-1, Shimadzu Scientific Instruments, Inc., 7102 Riverwood Dr., Columbia, MD 21046.

[6] Biolog EcoPlates™, 21124 Cabot Blvd., Hayward, CA 94545.

[7] Microtiter plate reader, VersaMax Microplate Reader, Molecular Devices, 1311 Orleans Dr., Sunnyvale, CA 94089.

[8] Black 96-well microplate, Costar®, Corning Incorporated, One Riverfront Plaza Corning, NY 14831.

[9] 4-Methylumbelliferone (MUB)-β-D-glucoside, Sigma-Aldrich, 3050 Spruce St., St. Louis, MO 63103.

[10] 4-MUB-*N*-acetyl-β-D-glucoside, Sigma-Aldrich, 3050 Spruce St., St. Louis, MO 63103.

[11] 4-MUB-phosphate, Sigma-Aldrich, 3050 Spruce St., St. Louis, MO 63103.

[12] Spectramax Gemini XS Spectrophotometer, Molecular Devices, 1311 Orleans Dr., Sunnyvale, CA 94089.

[13] Clear 96-well microplate, Costar®, Corning Incorporated, One Riverfront Plaza Corning, NY 14831.

[14] L-3,4-dihydroxyphenylalanine, Fisher Scientific, 300 Industry Dr., Pittsburgh, PA 15275.

[15] SAS version 9.2, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

[16] PC-ORD 6.12, MjM Software Design, PO Box 129, Gleneden Beach, OR 97388.

## LITERATURE CITED

Allison, S. D. and P. M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol. Biochem. 37: 937-944.

Breure, A. M. 2005. Ecological soil monitoring and quality assessment. Pages 281-305 in P. Doelman and H. J. P. Eijsackers, eds. Vital Soil, Function Value and Properties. Amsterdam: Elsevier.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17: 837-842.

Doran, J. W., M. Sarrantonio, and M. A. Liebig. 1996. Soil health and sustainability. Adv. Agron. 56: 1-54.

Garland, J. L. and A. L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57: 2351-2359.

German, D. P., M. N. Weintraub, A. S. Grandy, C. L. Lauber, Z. L. Rinkes, and S. D. Allison. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol. Biochem. 43: 1387-1397.

Haack, S. K., H. Garchow, M. J. Klug, and L. J. Forney. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Envir. Microbiol. 61: 1458-1468.

Hemphill, D. 2010. Oregon vegetables: lettuce. Available at http://horticulture.oregonstate.edu/content/lettuce-0. Access: May 1, 2011.

Hofrichter, M. 2002. Review: lignin conversion by manganese peroxidase (MnP). Enzyme Microb. Technol. 30: 454-466.

Insam, H. 2001. Developments in soil microbiology since the mid 1960s. Geoderma 100: 389-402.

Insam, H., C. C. Mitchell, and J. F. Dormaar. 1991. Relationship of soil microbial biomass and activity with fertilization practice and crop yield of three ultisols. Soil Biol. Biochem. 23: 459-464.

Jenkinson, D. S. 1976. The effects of biocidal treatments on metabolism in soil - IV. the decomposition of fumigated organisms in soil. Soil Biol. Biochem. 8: 203-208.

Jenkinson, D. S. and D. S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil - V. a method for measuring soil biomass. Soil Biol. Biochem. 8: 209-213.

Knapp, S. J. and J. M. Crane. 1995. Fatty acid diversity of section Inflexae *Limnanthes* (meadowfoam). Ind. Crops Prod. 4: 219-227.

Koch, A. L. 1985. The macroeconomics of bacterial growth. Pages 1-42 in M. Fletcher, G.D. Floodgate, eds. Bacteria in their Natural Environments. London: Academic Press.

McCune, B. and M. J. Mefford. 2011. PC-ORD. Gleneden Beach, OR: MjM Software Design.

Myrold, D. D. 2005. Transformations of nitrogen. Pages 333-372 in D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel, and D. A. Zuberer, eds. Principles and Applications of Soil Microbiology. Upper Saddle River, NJ: Pearson Education Inc.

Nannipieri, P., E. Kandeler, and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. Pages 1-33 in R. G. Burns and R. P. Dick, eds. Enzymes in the Environment. Marcel Dekker, NY: Marcel Dekker, Inc.

Purdy R. H. and C. D. Craig. 1987. Meadowfoam: new source of long-chain fatty acids. J. Am. Oil Chem. Soc. 64: 1493-1498.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/.

Schimel, J. P. and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol. Biochem. 35: 549-563.

Singler, V. 2004. Community level physiological profiling (CLPP). Available at http://www.eeescience.utoledo.edu/Faculty/Sigler/Von_Sigler/LEPR_Protocols_files/CLPP.pdf. Accessed: June 26, 2012.

Sinsabaugh, R. L. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42: 391-404.

Sinsabaugh, R. L., R. K. Antibus, A. E. Linkins, C. A. McClaugherty, L. Rayburn, D. Repert, and T. Weiland. 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecol. 74: 1586-1593.

Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60: 1-24.

Sinsabaugh, R. L., M. E. Gallo, C. Lauber, M. P. Waldrop, and D. R. Zak. 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75: 201-215.

Stevens, J. F., R. L., Reed, S. Alber, L. Pritchett, and S. Machado. 2009. Herbicidal activity of glucosinolate degradation products in fermented meadowfoam (*Limnanthes alba*) seed meal. J. Agric. Food Chem. 57: 1821-1826.

Stotzky, G. 1997. Quantifying the metabolic activity of microbes in soil. Pages 453-458 in C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter, eds. Manual of Environmental Microbiology. American Society for Microbiology. WA: ASM Press.

Stursova, M., C. Crenshaw, and R. Sinsabaugh. 2006. Microbial responses to long-term N deposition in a semiarid grassland. Microb. Ecol. 51: 90-98.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19: 703-707.

Vaughn, S. F., R. A. Boydston, and C. A. Mallory-Smith. 1996. Isolation and identification of (3-methoxyphenyl) acetonitrile as a phytotoxin from meadowfoam (*Limnanthes alba*) seed meal. J. Chem. Ecol. 22: 1939-1949.

Wallenstein, M. D. and M. N. Weintraub. 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biol. Biochem. 40: 2098-2106.

Wardle, D. A. and A. Ghani. 1995. A critique of the microbial metabolic quotient ($qCO_2$) as a bioindicator of disturbance and ecosystem development. Soil Biol. Biochem. 27: 1601-1610.

Zeglin, L., M. Stursova, R. Sinsabaugh, and S. Collins. 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154: 349-359.

Zaccardelli, M., D. Villecco, G. Celano, and R. Scotti. 2013. Soil amendment with seed meals: Short term effects on soil respiration and biochemical properties. Appl. Soil Ecol. 72: 225-231.

Table 5.1. Pearson's correlation coefficient between microbial biomass and soil enzyme activities in soil samples collected from meadowfoam seed meal amended and non-amended control[a].

| | MBC | MBN | BG | PHOS | NAG | PHENOX |
|---|---|---|---|---|---|---|
| | ---------- Expt 1 ---------- | | | | | |
| MBN | 0.803*** | | | | | |
| BG | 0.526*** | 0.494*** | | | | |
| PHOS | 0.530*** | 0.477*** | 0.723*** | | | |
| NAG | 0.663*** | 0.632*** | 0.744*** | 0.662*** | | |
| PHENOX | -0.187 | -0.442** | -0.054 | -0.029 | -0.363* | |
| PEROX | -0.776*** | -0.798*** | -0.511*** | -0.390** | -0.772*** | 0.423** |
| | ---------- Expt 2 ---------- | | | | | |
| MBN | 0.933*** | | | | | |
| BG | -0.082 | -0.041 | | | | |
| PHOS | 0.107 | 0.172 | 0.526*** | | | |
| NAG | 0.130 | 0.120 | 0.220 | 0.550*** | | |
| PHENOX | 0.055 | 0.007 | 0.162 | -0.103 | -0.396** | |
| PEROX | 0.067 | 0.087 | -0.003 | 0.083 | -0.549*** | 0.638*** |

[a] MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; BG = β-glucosidase; PHOS = acid phosphatase; NAG = β-*N*-acetylglucosaminidase; PHENOX = phenol oxidase; PEROX = peroxidase.

* Significant at $p < 0.05$.

** Significant at $p < 0.01$.

*** Significant at $p < 0.001$.

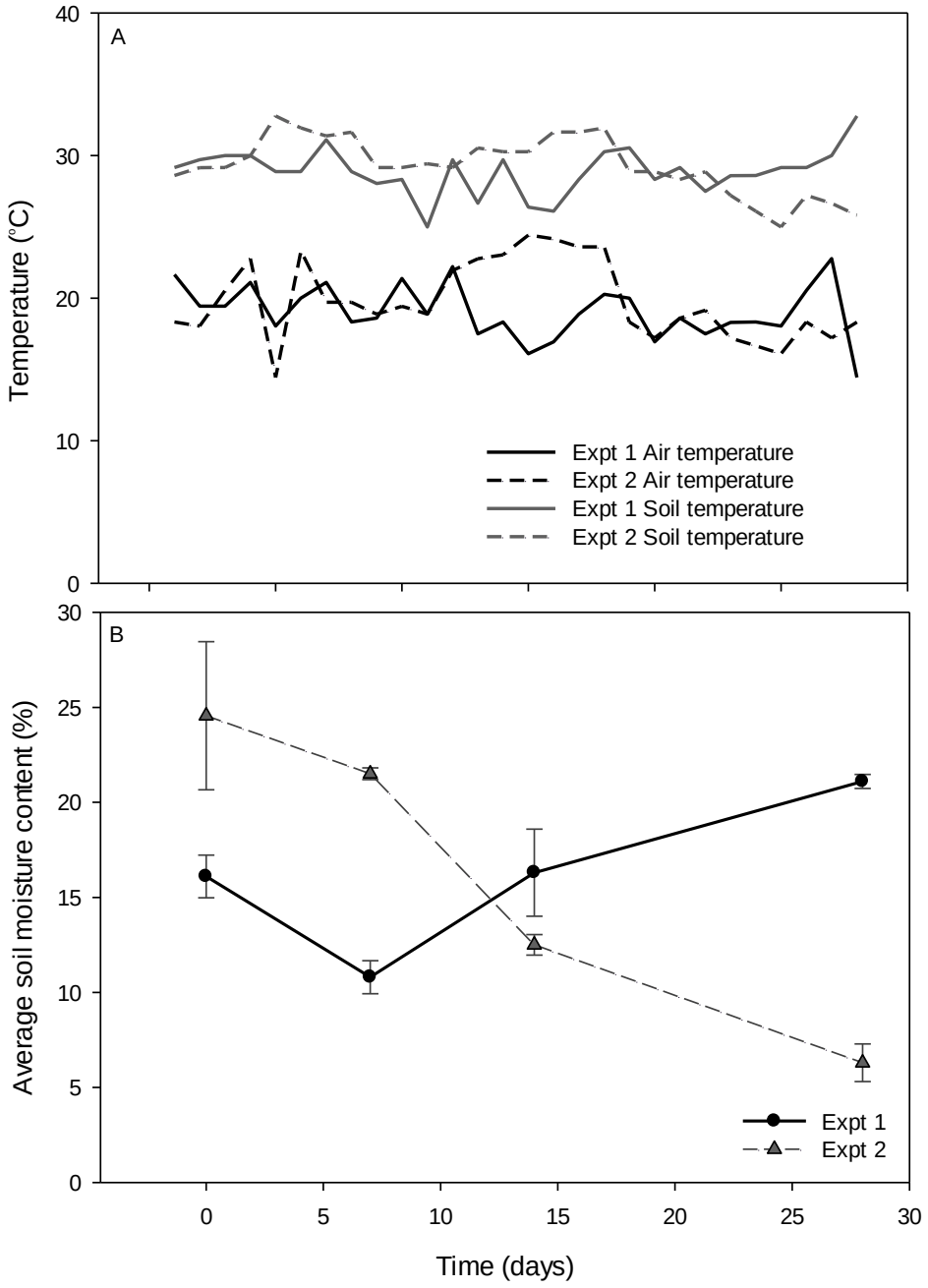



Figure 5.1. Environmental data of average daily air and top 5-cm soil depth temperatures recorded in 2012 at a weather station located in Hyslop Field Lab, Corvallis, Oregon, approximately 13 km from the study site (A) and average soil moisture at top 5-cm soil depth measured from study areas (B).

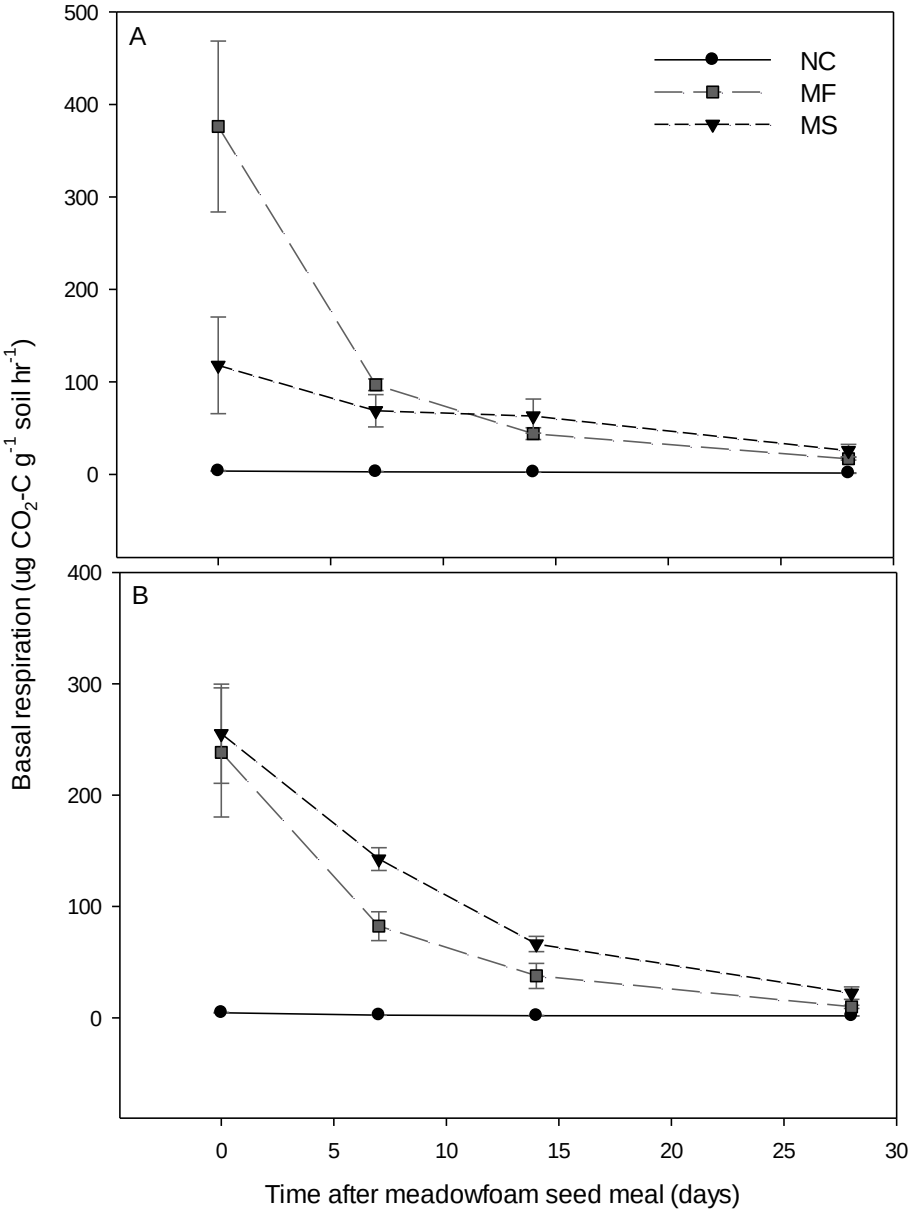



Figure 5.2. Total $CO_2$ of basal respiration after incubation in the dark for 48 hours at 25 ºC in Experiment 1 (A) and Experiment 2 (B). NC = non-amended; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

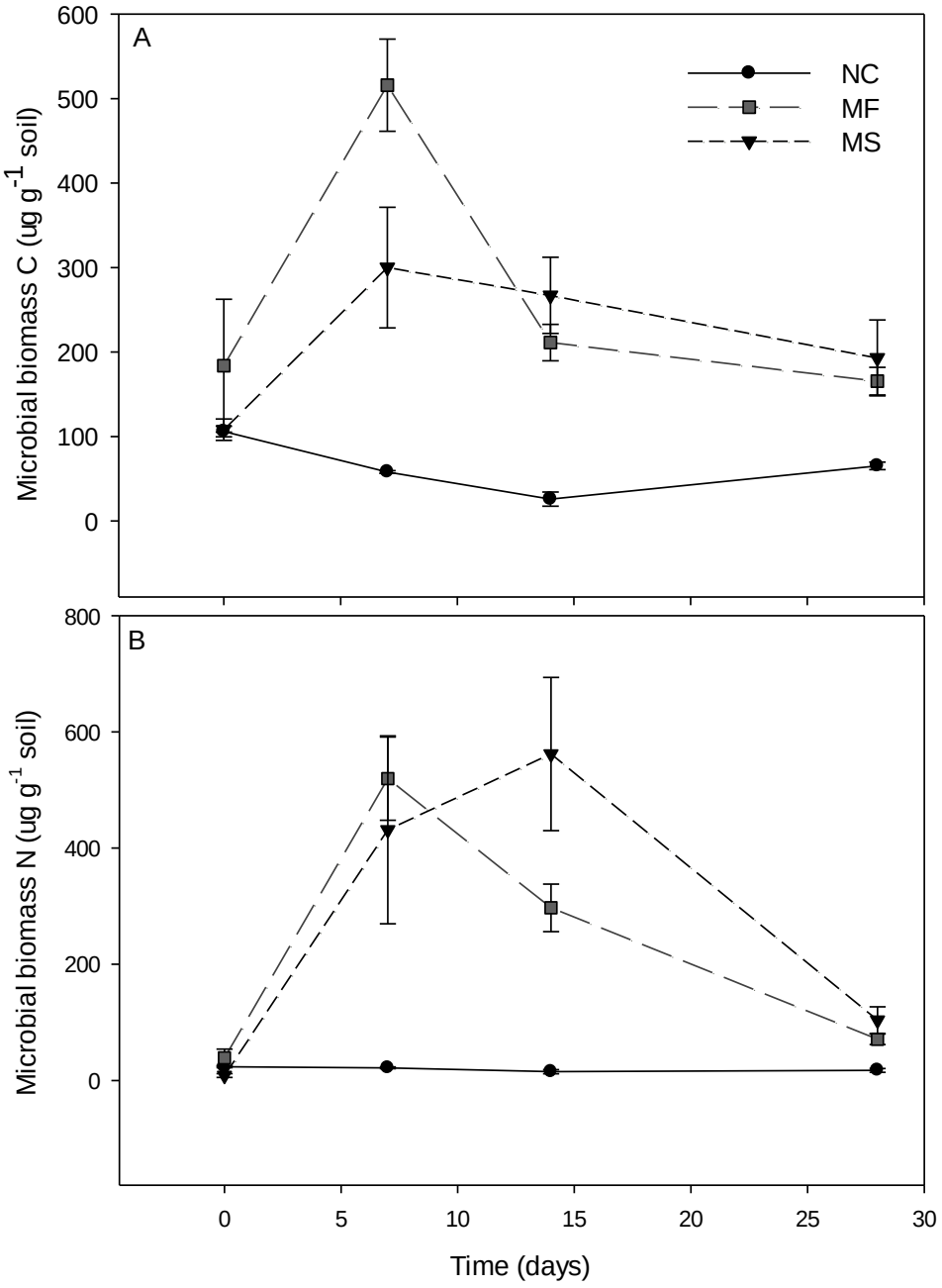



Figure 5.3. Microbial biomass carbon (A) and nitrogen (B) after 24-hour fumigation in Experiment 1. NC = non-amended; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

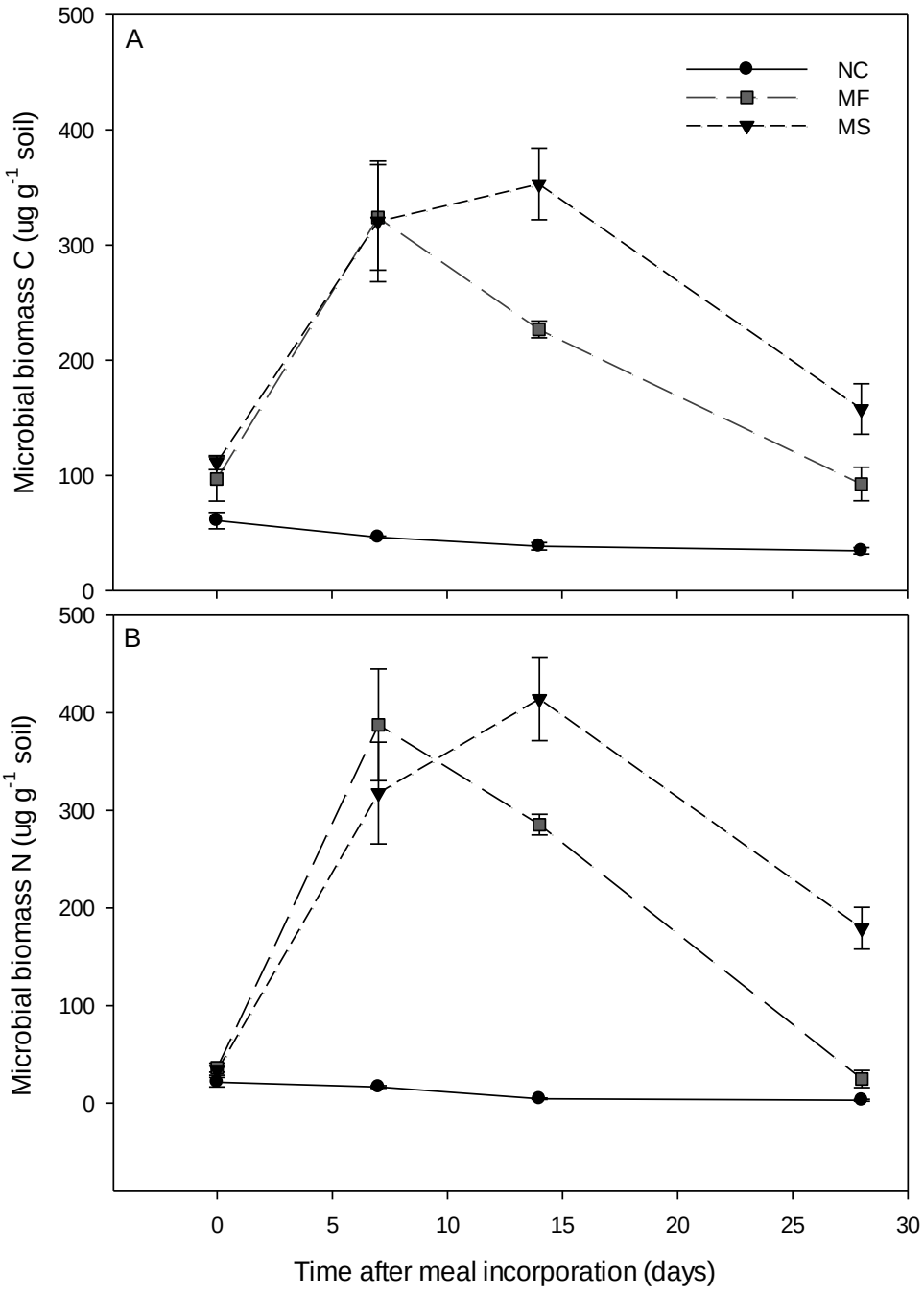



Figure 5.4. Microbial biomass carbon (A) and nitrogen (B) after 24-hour fumigation in Experiment 2. NC = non-amended; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

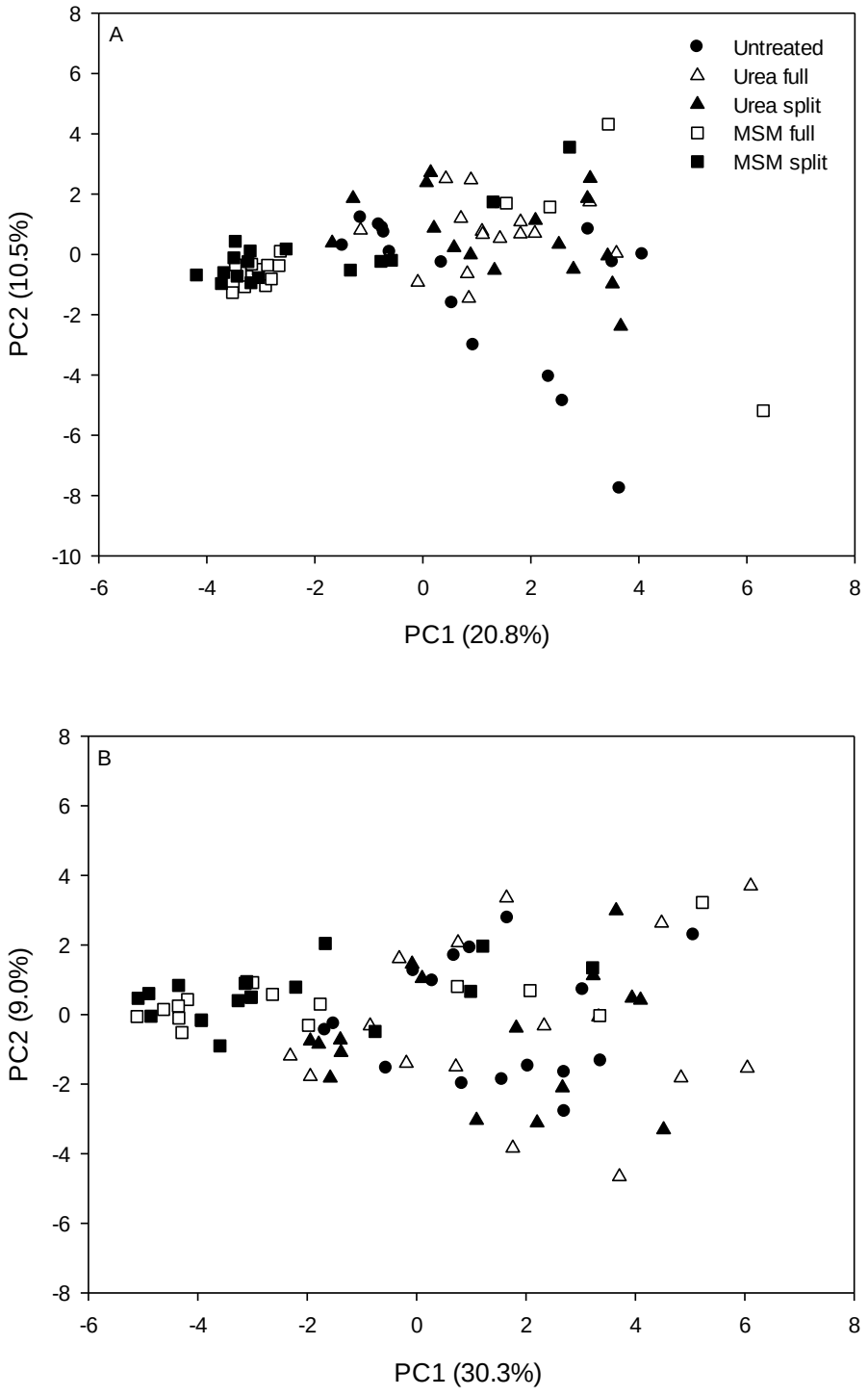



Figure 5.5. PC analysis of soil community-level physiological profiling (CLPP) using Biolog EcoPlates™ in Experiment 1 (A) and in Experiment 2 (B) at 72-hour incubation. See Appendix C.5 for high loading variables (Pearson, $r \geq |0.5|$) associated with each PC variable.

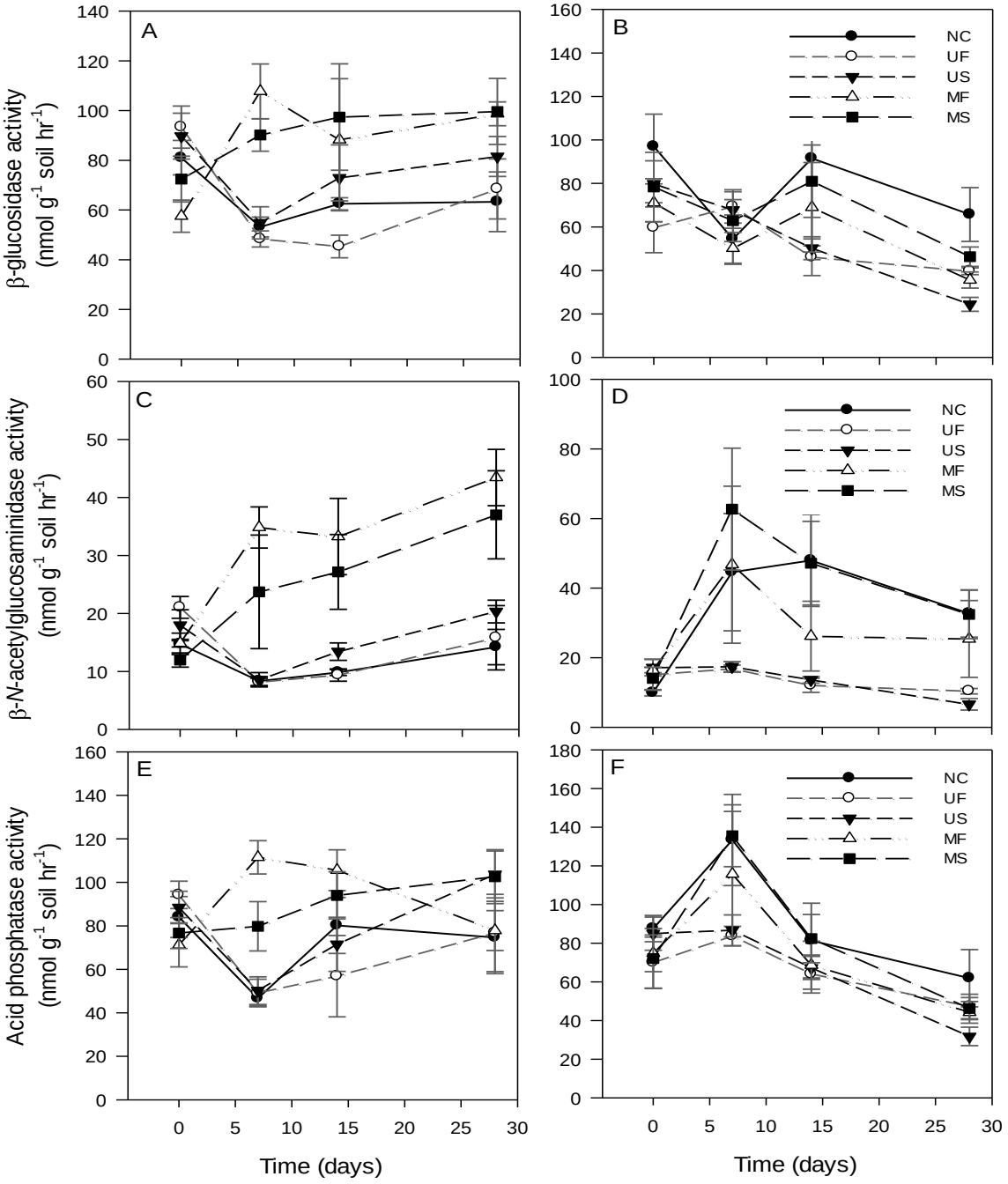



Figure 5.6. Hydrolytic enzyme activities from Experiment 1: β-glucosidase (A), β-N-acetylglucosaminidase (C), and acid phosphatase (E) and from Experiment 2: β-glucosidase (B), β-N-acetylglucosaminidase (D), and acid phosphatase assays (F). NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

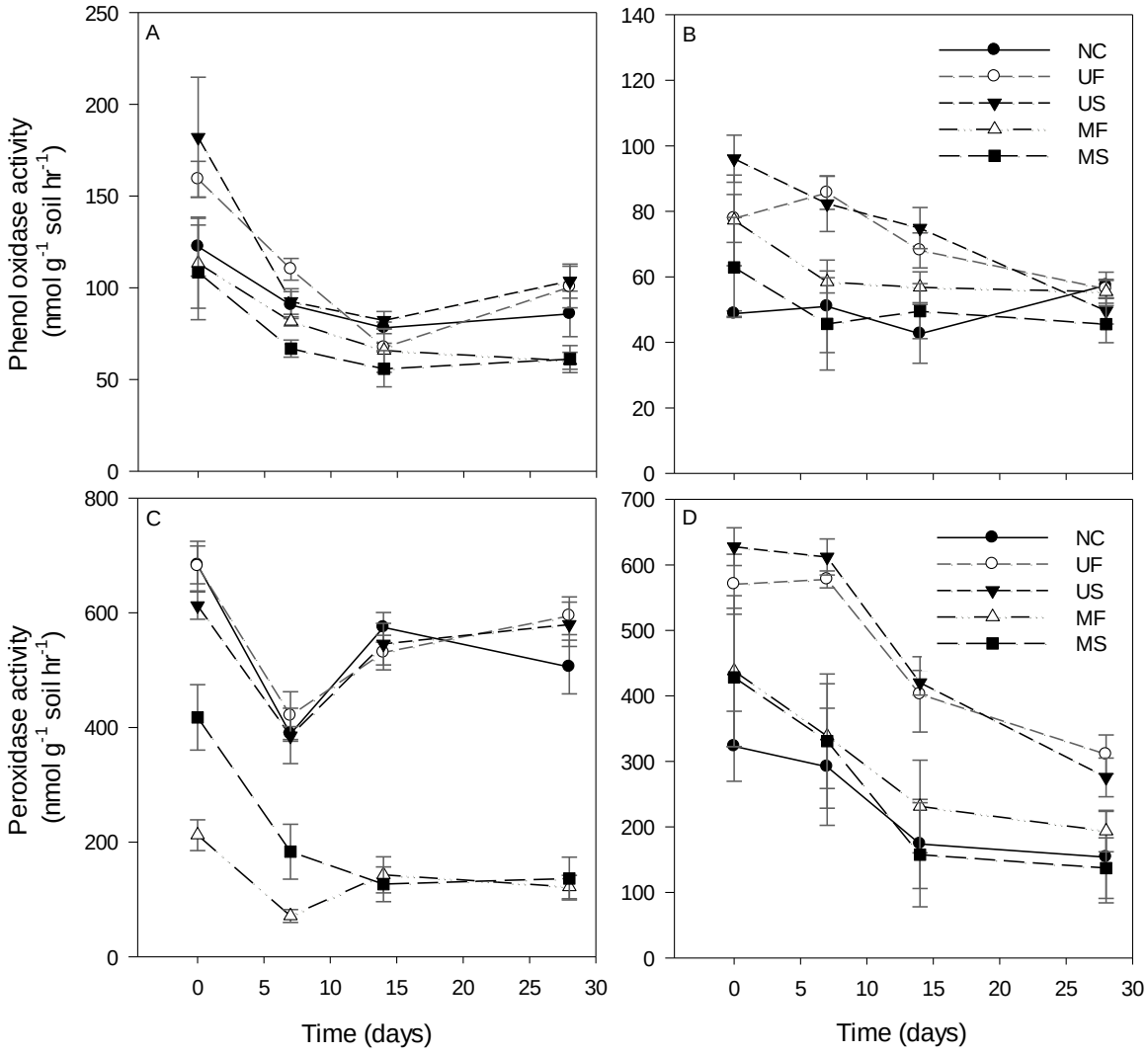



Figure 5.7. Oxidative enzyme activities from Experiment 1: phenol oxidase (A) and peroxidase (C) and Experiment 2: phenol oxidase (B) and peroxidase (D). NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.

# CHAPTER 6

## GENERAL CONCLUSIONS

These studies were conducted to evaluate the effectiveness of meadowfoam seed meal (MSM) for weed control and to determine optimal timing, rate, and application method at the field scale. Analyses were done on the effects of MSM on the crop growth, weed emergence and growth, the temporal change in biodegradation of glucosinolate glucolimnanthin (GLN) and glucosinolate breakdown products (GBPs) and the change on soil microbial composition and function. The studies were first conducted in the laboratory and the greenhouse (Chapter 2), then expanded to field trials (Chapter 3, 4, and 5).

The herbicidal effect in MSM was compared to other Brassicaceae seed meals in Petri dish assays and was evaluated in the greenhouse when applied as a soil amendment (Chapter 2). Non-activated MSM contained only ground MSM, while activated MSM was prepared by adding 1% by weight of freshly ground meadowfoam seeds to MSM in order to provide active myrosinase as recommended by Stevens et al. (2009). Activated MSM and brown mustard (*Brassica juncea* (L.) Czern.) meal yielded greater suppression of lettuce emergence and growth than camelina (*Camelina sativa* (L.) Crantz) meal and non-activated MSM. The greater glucosinolate concentration and different glucosinolate structure were possible driving factors. However, the effectiveness of MSM depended on the activation process. Myrosinase enzyme and water are needed to facilitate the hydrolysis of GLN to GBPs. Among detected GBPs, isothiocyanate had the most herbicidal effect, followed by MPAA and nitrile.

Studies with soil amended with activated MSM were conducted to evaluate whether activation was necessary to enhance phytotoxicity of MSM. In greenhouse studies, activated-MSM inhibited lettuce emergence and growth greater than non-activated MSM treatments (Chapter 2). The effects of activation method on weed emergence and growth were evaluated in field trials (Chapter 3). Weed aboveground biomass was harvested 64 days after MSM incorporation. No difference in weed emergence and growth was observed between activated and non-activated MSM. Both herbicide and fertilizer effects from MSM-amended treatments were observed. These combined of effects made it difficult to evaluate differences between MSM forms. Isothiocyanate concentrations were detected in activated MSM but not in non-activated MSM. There was a difference in effectiveness between activated and non-activated MSM in the Petri dish and greenhouse studies and the presence of a potent herbicidal compound (isothiocyanate) make activated MSM as a good candidate for further evaluation (Chapter 4).

Greenhouse studies were used to test the effect of planting time for crop safety (Chapter 2). Lettuce seeds were sown on different planting dates, 0, 6, and 12 days after 3% MSM incorporation. On 0 day after incorporation (DAI), the inhibition of lettuce germination in activated MSM was observed for six days, while no emergence suppression was observed when planting on 6 or 12 DAI. There was a fertilizer effect from MSM addition on planting dates of 6 and 12 DAI, especially in non-activated MSM. The phytotoxicity lasted a maximum of 6 days in 3% activated MSM. In field studies (Chapter 3), lettuce seedlings were transplanted on 7 DAI to prevent residual crop injury.

At harvest, no injury was observed on transplanted lettuce plants. Lettuce biomass and leaf nitrogen contents were greater in MSM-amended compared to control plots.

Different concentrations (3, 5, and 7% by weight) of MSM were applied as soil amendments in the field (Chapter 3). After MSM incorporation, water was applied to facilitate the hydrolysis of GLN. Weed emergence was inhibited at least 28% for 3% MSM and at least 54% for 5 and 7% MSM compared to the control. There was no difference in weed biomass between the control and MSM amendments due to the fertilizer effect. Isothiocyanate, with potent herbicidal activity was observed only in 5 and 7% activated MSM treatments. Differences among plant life forms were found in response to MSM concentrations. The plots with abundant annual or monocot species such as barnyardgrass, common purslane, and wild garlic, tended to have fewer perennial plants. In early summer, plots treated with 3 and 5% MSM had more annual and monocot plants. In late summer, plots treated with 3, 5, and 7% of MSM had more monocot plants. No herbicidal selectivity of MSM was detected for a particular weed species. Based on biomass data, the fertilizer effect overrode the herbicide effect. In order to utilize the herbicidal activity of MSM, the application rate should be between 3 and 7% by weight and incorporated close to transplanted crops using a method such as band application. Application should be done at the beginning of crop establishment to provide weed suppression and avoid too much fertilizer availability for later emerging weeds. A fast establishing crop may take advantage of organic materials added from MSM application.

The impacts of split and single applications of activated MSM on weeds were evaluated (Chapter 4). Activated MSM was applied at 2.86 kg $m^{-2}$ which resulted in a nitrogen rate of at least 168 kg $ha^{-1}$, the recommended rate for leaf lettuce. The single

MSM application was applied at a full rate on day 0. The split MSM application contained the same total amount as the single application with half applied on day 0 and 7. In addition to MSM-amended and non-amended plots, urea was used as a mineral nitrogen source in order to account for the fertilizer effect of the MSM, and applied at 168 kg $ha^{-1}$ on the same schedule as MSM. The split application was tested because the herbicide effect may decrease after 6 days. Johnson-Maynard et al. (2005) suggested that the reapplication of seed meal may provide adequate weed control throughout the growing season but could increase late-season weed biomass due to the increase in plant-available nitrogen. The organic nitrogen supplied from MSM was plant available at a similar or greater level compared to urea. Weed emergence and growth were inhibited in MSM applications. Activated MSM inhibited spiny sowthistle (*Sonchus asper* (L.) Hill) greater than 95% for emergence and 85% for biomass compared to the untreated control. In late summer, crop injury was observed in the split MSM application possibly due to high soil moisture and low soil temperature conditions during the time of transplanting and the second MSM application. Greater soil moisture and lower temperature were ideal for the GLN hydrolysis. The split MSM application provided a weed control similar to the full MSM application but application timing and concentration need to be adjusted to prevent residual crop injury. For ease in use, a single MSM application as a pre-emergence soil amendment is recommended to increase crop yield and weed suppression.

Effects of MSM on soil quality were analyzed; specifically, the change in composition and function of soil microorganisms were investigated (Chapter 5). MSM provided a carbon source for soil microbes with greater basal respiration with MSM application compared to urea-amended and non-amended soils. The carbon inputs

increased the gross metabolic activity of the mixed microbial population. Soil microbes reallocated carbon to biomass and enzyme production. The reallocation occurred within 7 to 14 days after the initial MSM application. Microbial biomass increased at least 80% for carbon and 95% for nitrogen compared to the non-amended control. β-*N*-acetylglucosaminidase activity was highly correlated with microbial biomass nitrogen and involved in the acquisition of nitrogen from organic sources. Microbial communities in MSM-amended treatments utilized the complex carbon sources, amine, polymer, and phenolic compounds, to a relatively greater degree than microbial communities in urea-amended or non-amended treatments. The microbial biomass accumulation and enzyme activity increased 7 and 14 days after the initial MSM application, possibly because the toxic effects due to the release of GBPs in MSM application were transient. These short-term results cannot be extended to the potential effects of MSM on soil microbial community and function in other areas. The effect of MSM application on soil quality needs a longer timespan study.

Temporal changes in biodegradation of GLN and GBPs in MSM-amended soil studies were conducted (Chapter 2, 3, and 4). The effects of each GBP on the germination and growth of lettuce (used as bioassay plant) were determined (Chapter 2). The detectable GBPs were 3-methoxybenzyl isothiocyanate (isothiocyanate), 3-methoxyphenylacetonitrile (nitrile), and 3-methoxyphenylacetic acid (MPAA). MPAA, a previously unknown metabolite, was identified in our study using LC-UV and high resolution LC-MS/MS comparisons with a standard MPAA. There was no MPAA detected in either MSM or activated MSM without soil being present. Soil incubation with nitrile confirmed the nitrile as a parent compound of MPAA. Soil microbes may

possibly produce nitrilase and catalyze nitrile hydrolysis to MPAA. With the presence of myrosinase in activated MSM, GLN was hydrolysed and converted into isothiocyanate within 24 hours. No isothiocyanate was detected in non-activated MSM possibly because soil microorganisms in this soil did not produce sufficient myrosinase. Isothiocyanate was the most effective GBPs in inhibition of lettuce emergence and growth followed by MPAA and nitrile. The inhibition was greater for radicle length than hypocotyl length. Isothiocyanate had a short-half life and was degraded by 72 hours. The rapid degradation was confirmed when MSM was applied as a soil amendment in the field studies. Although the nitrile and MPAA were detectable for 12 to 18 days in the centrifuge tubes (Chapter 2) and 9 days in the field (Chapter 3 and 4), their concentrations may not be sufficient for herbicidal activity.

The rapid degradation of bioactive compounds in the soil should allow the use of activated MSM preplant for weed control if there is an adequate delay before planting the crop to prevent residual crop injury. The early summer MSM application provided better weed control than the late summer MSM application possibly due to less weed pressure in the early summer and appropriate environmental conditions for GLN hydrolysis. Short season and/or transplanted crops are recommended because good stand establishment helps to protect the crop from remaining allelochemicals, increases competitiveness to late-season emerged weeds, and readily uses available plant nutrients from the degraded MSM. MSM treatments applied at 2.86 kg $m^{-2}$ and soil incorporated promoted lettuce growth, provided a nitrogen source equivalent to mineral nitrogen fertilizer, and inhibited weed emergence and growth.

In organic farming systems, weed control options are limited. Bioherbicides from natural plant products are desired but the development of these products face many difficulties including degradation, efficacy, selectivity, and uptake by target plants. In developing activated MSM, the optimization ratio between ground meadowfoam seed to MSM needs further investigation in order to reduce the amount of MSM that needs to be applied. Further study of synergistic, antagonistic, or additive responses among GBP combinations for herbicidal activity are also important. The benefits of MSM application on soil quality due to basic changes of microbial communities need further study to detect the effects over a longer timespan. Exploration in carbon and nitrogen mineralization would help in understanding the conversion of MSM organic materials for soil fertility and productivity. We did not include economic evaluation in our study and this needs to be explored.

The results from this study improve our understanding about the use of MSM as a soil amendment for pre-emergent herbicidal activity, and the effects of MSM on crop growth and soil microbial composition and function. The study benefits meadowfoam growers as well as provides an alternative bioherbicide for use in organic farms, the production of high value crops, and the cultivation of plants for which less herbicide application is desired. Because the bioherbicide effect was transient, MSM also could be considered as carbon and nitrogen sources.

# BIBLIOGRAPHY

AACC International. 2009. Approved methods of the AACC (11th ed). St. Paul, MN: American Association of Cereal Chemists (Method 56-11.02).

Allison, S. D. and P. M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol. Biochem. 37: 937-944.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral. Ecol. 26: 32-46.

Bartelt, R. J. and K. L. Mikolajczak. 1989. Toxicity of compounds derived from *Limnanthes alba* seed to fall armyworm (Lepidoptera: Noctuidae) and European corn borer (Lepidoptera: Pyralidae) Larvae. J. Econ. Entomol. 82: 1054-1060.

Borek, V. and M. J. Morra. 2005. Ionic thiocyanate ($SCN^-$) production from 4-hydroxybenzyl glucosinolate contained in *Sinapis alba* seed meal. J. Agric. Food Chem. 53: 8650-8654.

Borek, V., M. J. Morra, P. D. Brown, and J. P. McCaffrey. 1995. Transformation of the glucosinolate-derived allelochemicals allylisothiocyanate and allylnitrile in soil. J. Agric. Food Chem. 43: 1935-1940.

Borek, V., M. J. Morra, and J. P. McCaffrey. 1996. Myrosinase activity in soil extracts. Soil Sci. Soc. Am. J. 60: 1792-1797.

Brady, D., A. Beeton, J. Zeevaart, C. Kgaje, F. Rantwijk, and R. A. Sheldon. 2004. Characterisation of nitrilase and nitrile hydratase biocatalytic systems. Appl. Microbiol. Biotechnol. 64: 76-85.

Breure, A. M. 2005. Ecological soil monitoring and quality assessment. Pages 281-305 in P. Doelman and H. J. P. Eijsackers, eds. Vital Soil, Function Value and Properties. Amsterdam: Elsevier.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17: 837-842.

Brown, P. D. and M. J. Morra. 1995. Glucosinolate-containing plant tissues as bioherbicides. J. Agric. Food Chem. 43: 3070-3074.

Brown, P. D. and M. J. Morra. 1996. Hydrolysis products of glucosinolates in *Brassica napus* tissues as inhibitors of seed germination. Plant Soil 181: 307-316.

Brown, J. and M. J. Morra. 2005. Glucosinolate-containing seed meal as a soil amendment to control plant pests. Subcontract Report 2000-2002, Univ. of Idaho, Moscow, Idaho. 99 p.

Burg, D. A. and R. Kleiman. 1991. Preparation of meadowfoam dimer acids and dimer esters and their use as lubricants. J. Am. Oil Chem. Soc. 68:600-603.

Doran, J. W., M. Sarrantonio, and M. A. Liebig. 1996. Soil health and sustainability. Adv. Agron. 56: 1-54.

Ehrensing, D. T. and S. O. Guy. 2008. Oil seed crops: camelina. Oregon State University Extension Service. EM 8953-E.

Ehrensing, D. T., G. D. Jolliff, J. M. Crane, and R. S. Karow. 1997. Growing meadowfoam in the Willamette Valley. Oregon State University Extension Service. EM 8567.

Fahey, J. W., A. T. Zalcmann, and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56: 5-51.

Fenwick, G. R., R. K. Heaney, and W. J. Mullin. 1982. Glucosinolates and their breakdown products in food and food plants. Crit. Rev. Food. Sci. 18: 123-201.

Gardiner, J. B., M. J. Morra, C. V. Eberlein, P. D. Brown, and V. Borek. 1999. Allelochemicals released in soil following incorporation of rapeseed (*Brassica napus*) green manures. J. Agric. Food Chem. 47: 3837-3842.

Garland, J. L. and A. L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57: 2351-2359.

Gaudet, C. L. and P. A. Keddy. 1995. Competitive performance and species distribution in shortline plant communities: a comparative approach. Ecol. 76: 280-291.

German, D. P., M. N. Weintraub, A. S. Grandy, C. L. Lauber, Z. L. Rinkes, and S. D. Allison. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol. Biochem. 43: 1387-1397.

Gimsing, A. and J. Kirkegaard. 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochem. Rev. 8: 299-310.

Haack, S. K., H. Garchow, M. J. Klug, and L. J. Forney. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Envir. Microbiol. 61: 1458-1468.

Handiseni, M., J. Brown, R. Zemetra, and M. Mazzola. 2011. Herbicidal activity of Brassicaceae seed meal on wild oat (*Avena fatua*), Italian ryegrass (*Lolium multiflorum*), redroot pigweed (*Amaranthus retroflexus*), and prickly lettuce (*Lactuca serriola*). Weed Technol. 25: 127-134.

Hansson, D., M. J. Morra, V. Borek, A. J. Snyder, J. L. Johnson-Maynard, and D. C. Thill. 2008. Ionic thiocyanate ($SCN^-$) production, fate, and phytotoxicity in soil amended with Brassicaceae seed meals. J. Agric. Food Chem. 56: 3912-3917.

Hemphill, D. 2010. Oregon vegetables: lettuce. Available at http://horticulture.oregonstate.edu/content/lettuce-0. Access: May 1, 2011.

Hirsinger, F. 1989. New annual oil crops. Pages 518-532 in G. Röbbelen, R. K. Downey, and A. Ashri, eds. Oil Crops of the World. New York: McGraw-Hill.

Hofrichter, M. 2002. Review: lignin conversion by manganese peroxidase (MnP). Enzyme Microb. Technol. 30: 454-466.

Horneck, D. A., J. M. Hart, K. Topper, and B. Koepsell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Corvallis, OR: Agricultural Experiment Station, SM 89:4. 21 p.

Insam, H. 2001. Developments in soil microbiology since the mid 1960s. Geoderma 100: 389-402.

Insam, H., C. C. Mitchell, and J. F. Dormaar. 1991. Relationship of soil microbial biomass and activity with fertilization practice and crop yield of three ultisols. Soil Biol. Biochem. 23: 459-464.

Jenkinson, D. S. 1976. The effects of biocidal treatments on metabolism in soil - IV. the decomposition of fumigated organisms in soil. Soil Biol. Biochem. 8: 203-208.

Jenkinson, D. S. and D. S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil - V. a method for measuring soil biomass. Soil Biol. Biochem. 8: 209-213.

Johnson-Maynard, J. M., Morra, L. Dandurand, C. William, and M. J. Butters. 2005. Brassicaceae seed meal application for weed reduction and improved nitrogen management in organic farming systems. Project report, University of Idaho, Moscow, ID.

Kakani, R., J. Fowler, A.-U. Haq, E. Murphy, T. Rosenberger, M. Berhow, and C. A. Bailey. 2012. Camelina meal increases egg n-3 fatty acid content without altering quality or production in laying hens. Lipids 47: 519-526.

Kleiman, R. 1990. Chemistry of new industrial oilseed crops. Pages 198-199 in J. Janick and J. E. Simon, eds. Advances in New Crops. Portland, OR: Timber Press.

Knapp, S. J. and J. M. Crane. 1995. Fatty acid diversity of section Inflexae *Limnanthes* (meadowfoam). Ind. Crops Prod. 4: 219-227.

Koch, A. L. 1985. The macroeconomics of bacterial growth. Pages 1-42 in M. Fletcher, G.D. Floodgate, eds. Bacteria in their Natural Environments. London: Academic Press.

Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika 29: 115-129.

Linderman, R. G., E. A. Davis, and C. J. Masters. 2007. Response of conifer seedlings to potting medium amendment with meadowfoam seed meal. Pages 138-142 in J. Janick and A. Whipkey, eds. Issues in New Crops and New Uses. Alexandria, VA: ASHS Press.

Machado, S. 2007. Allelopathic potential of various plant species on downy brome: implications for weed control in wheat production. Agron. J. 99: 127-132.

Macías, F. A., D. Castellano, and J. M. G. Molinillo. 2000. Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. J. Agric. Food Chem. 48: 2512-2521.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. Cancer Res. 27: 209-220.

Martens, M-H. and K. Martens. 2002. Organic weed control: cultural and mechanical methods. Acres U.S.A. 32: 1-7.

Marx, E. S., J. Hart, and R. G. Stevens. 1999. Soil test interpretation guide. Oregon State University Extension Service. EC 1478.

Mather, P. M. 1976. Computational methods of multivariate analysis in physical geography. London: John Wiley & Sons. 532 p.

Matthiessen, J. and J. Kirkegaard. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Crit. Rev. Plant Sci. 25: 235-265.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. Gleneden Beach, OR: MjM Software Design. 300 p.

McCune, B. and M. J. Mefford. 2011. PC-ORD. Gleneden Beach, OR: MjM Software Design.

Meyer, S. L. F., I. A. Zasada, S. B. Orisajo, and M. J. Morra. 2011. Mustard seed meal mixtures: management of *Meloidogyne incognita* on pepper and potential phytotoxicity. J. Nematol. 43: 7-15.

Monaco, T. J., S. C. Weller, and F. M. Ashton. 2002. Weed Science, Principles and Practices. 4th ed. New York: John Wiley and Sons, Inc. 671 p.

Morra, M. J. and J. A. Kirkegaard. 2002. Isothiocyanate release from soil-incorporated *Brassica* tissues. Soil Biol. Biochem. 34: 1683-1690.

Muller, B. and E. Garnier. 1990. Components of relative growth rate and sensitivity to nitrogen availability in annual and perennial species of *Bromus*. Oecologia 84: 513-518.

Myrold, D. D. 2005. Transformations of nitrogen. Pages 333-372 in D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel, and D. A. Zuberer, eds. Principles and Applications of Soil Microbiology. Upper Saddle River, NJ: Pearson Education Inc.

Nannipieri, P., E. Kandeler, and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. Pages 1-33 in R. G. Burns and R. P. Dick, eds. Enzymes in the Environment. Marcel Dekker, NY: Marcel Dekker, Inc.

Nelson, D. W. and L. E. Sommers. 1996. Total carbon, organic carbon, and organic matter. Pages 961-1010 in D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, M. E. Sumner, eds. Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Ser. 5. Madison, WI: SSSA.

O'Reilly, C. and P. D. Turner. 2003. A review: the nitrilase family of CN hydrolyzing enzymes - a comparative study. J. Appl. Microbiol. 95: 1161-1174.

Purdy, R. H. and C. D. Craig. 1987. Meadowfoam: new source of long-chain fatty acids. J. Am. Oil Chem. Soc. 64: 1493-1498.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/.

Rakariyatham, N., B. Butrindr, H. Niamsup, and L. Shank. 2005. Screening of filamentous fungi for production of myrosinase. Brazilian J. Microbiol. 36: 242-245.

Rice, A. R., J. L. Johnson-Maynard, D. C. Thill, and M. J. Morra. 2007. Vegetable crop emergence and weed control following amendment with different Brassicaceae seed meals. Renew. Agric. Food Syst. 22: 204-212.

Ritz, C. and J. C. Streibig. 2005. Bioassay Analysis using R. J. Statist. Software. 12: 1-22.

Ritz, C. and J. C. Streibig. 2012. Dose response curves and other nonlinear curves in Weed Science and Ecotoxicology with the add-on package drc in R. Available at http://www.bioassay.dk/index-filer/start/DraftDrcManual.pdf. Accessed: May 26, 2013.

Rosa, E. A. S., R. K. Heaney, G. R. Fenwick, and C. A. M. Portas. 1997. Glucosinolates in crop plants. Pages 99-215 in J. Janick, ed. Horticultural Reviews. Hoboken, NJ: John Wiley & Sons, Inc.

Rösch, H., M. W. V. Rooyen, and G. K. Theron. 1997. Predicting competitive interactions between pioneer plant species by using plant traits. J. Veg. Sci. 8: 489-494.

Russell, D. H. and R. D. Edmondson. 1997. High-resolution mass spectrometry and accurate mass measurements with emphasis on the characterization of peptides and proteins by matrix-assisted laser desorption/ionization time-of-fight mass spectrometry J. Mass Spec. 32: 263-276.

Sakorn, P., N. Rakariyatham, H. Niamsup, and P. Nongkunsarn. 2002. Rapid detection of myrosinase-producing fungi: a plate method based on opaque barium sulphate formation. World J. Microbiol. Biotech. 18: 73-74.

Schimel, J. P. and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol. Biochem. 35: 549-563.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Tech. 9: 218-227.

Singler, V. 2004. Community level physiological profiling (CLPP). Available at http://www.eeescience.utoledo.edu/Faculty/Sigler/Von_Sigler/LEPR_Protocols_files/CLPP.pdf. Accessed: June 26, 2012.

Sinsabaugh, R. L. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42: 391-404.

Sinsabaugh, R. L., R. K. Antibus, A. E. Linkins, C. A. McClaugherty, L. Rayburn, D. Repert, and T. Weiland. 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecol. 74: 1586-1593.

Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60: 1-24.

Sinsabaugh, R. L., M. E. Gallo, C. Lauber, M. P. Waldrop, and D. R. Zak. 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75: 201-215.

Steiner, J. J., G. W. Mueller-Warrant, S. M. Griffith, G. M. Banowetz, and G. W. Whittaker. 2006. Conservation practices in western Oregon perennial grass seed systems: II. meadowfoam rotation crop management. Agron. J. 98: 1501-1509.

Stevens, J. F., R. L., Reed, S. Alber, L. Pritchett, and S. Machado. 2009. Herbicidal activity of glucosinolate degradation products in fermented meadowfoam (*Limnanthes alba*) seed meal. J. Agric. Food Chem. 57: 1821-1826.

Stotzky, G. 1997. Quantifying the metabolic activity of microbes in soil. Pages 453-458 in C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter, eds. Manual of Environmental Microbiology. American Society for Microbiology. WA: ASM Press.

Streibig, J. C., M. Rudemo, and J. E. Jensen. 1993. Dose-response curves and statistical models. Pages 29-53 in J. C. Streibig and P. Kudsk, eds. Herbicide Bioassay. Boca Raton, FL: CRC Press.

Stursova, M., C. Crenshaw, and R. Sinsabaugh. 2006. Microbial responses to long-term N deposition in a semiarid grassland. Microb. Ecol. 51: 90-98.

Sullivan, D. M. and C. G. Cogger. 2003. Post-harvest soil nitrate testing for manured cropping systems, west of the Cascades. Oregon State University Extension Service. EM 8832-E.

Tani, N., M. Ohtsuru, and T. Hata. 1974. Purification and general characteristics of bacterial myrosinase produced by *Enterobacter cloacae*. Agr. Biol. Chem. 38: 1623-1630.

Thangstad, O. P., K. Evjen, and A. Bones. 1991. Immunogold-EM localization of myrosinase in Brassicaceae. Protoplama 161: 85-93.

Tripathi, M. K. and A. S. Mishra. 2007. Glucosinolates in animal nutrition: a review. Anim. Feed Sci. Technol. 132: 1-27.

USDA, NRCS. 2012. The PLANTS Database. Available at http://plants.usda.gov. Accessed: February 13, 2012.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19: 703-707.

VanEtten, C. H. and H. L. Tookey. 1978. Glucosinolates in cruciferous plants. Page 507-520 in R. F. Keeler, K. R. Van Kampen, and L. F. James, eds. Effects of Poisonous Plants on Livestock. New York: Academic Press.

Vaughn, S. F. 1999. Glucosinolates as natural pesticides. Pages 81-91 in: H. G. Cutler and S. J. Cutler, eds. Biologically Active Natural Products: Agrochemicals. New York: CRC Press.

Vaughn, S. F., M. A. Berhow, and B. Tisserat. 2008. Stimulation of plant growth by (3-methoxyphenyl) acetonitrile applied as a foliar spray *in vivo* or as a medium amendment *in vitro*. HortSci. 43: 372-375.

Vaughn, S. F., R. A. Boydston, and C. A. Mallory-Smith. 1996. Isolation and identification of (3-methoxyphenyl) acetonitrile as a phytotoxin from meadowfoam (*Limnanthes alba*) seed meal. J. Chem. Ecol. 22: 1939-1949.

Vaughn, S. F., D. E. Palmquist, S. M. Duval, and M. A. Berhow. 2006. Herbicidal activity of glucosinolate-containing seedmeals. Weed Sci. 54: 743-748.

Wallenstein, M. D. and M. N. Weintraub. 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biol. Biochem. 40: 2098-2106.

Wardle, D. A. and A. Ghani. 1995. A critique of the microbial metabolic quotient ($qCO_2$) as a bioindicator of disturbance and ecosystem development. Soil Biol. Biochem. 27: 1601-1610.

Zaccardelli, M., D. Villecco, G. Celano, and R. Scotti. 2013. Soil amendment with seed meals: Short term effects on soil respiration and biochemical properties. Appl. Soil Ecol. 72: 225-231.

Zangerl, A. R. and F. A. Bazzaz. 1983. Responses of an early and a late successional species of *Polygonum* to variations in resource availability. Oecologia 56: 397-404.

Zasada, I. A., J. E. Weiland, R. L. Reed, and J. F. Stevens. 2012. Activity of meadowfoam (*Limnanthes alba*) seed meal glucolimnanthin degradation products against soilborne pathogens. J. Agric. Food Chem. 60: 339-345.

Zeglin, L., M. Stursova, R. Sinsabaugh, and S. Collins. 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154: 349-359.

# APPENDIX A

**Appendix A.1:** Life form, plot frequency in each experiment of plant species observed in the sample plots at Lewis-Brown Horticulture Research Farm (43º 33′ 24″ N, 123º 13′ 7″ W), Oregon State University, Corvallis, OR in summer 2011. Life form codes: AD = annual dicot; AM = annual monocot; BD = biennial dicot; PD = perennial dicot; PM = perennial monocot.

| Bayer code | Family | Species | Common name | Life form | Frequency[a] | |
|---|---|---|---|---|---|---|
| | | | | | Expt1 | Expt2 |
| ALLVI | Liliaceae | *Allium vineale* L. | wild garlic | PM | 15 | 27 |
| AMARE | Amaranthaceae | *Amaranthus retroflexus* L. | redroot pigweed | AD | 26 | 8 |
| ANGAR | Caryophyllaceae | *Anagallis arvensis* L. | scarlet pimpernel | AD | 1 | 0 |
| CAPBP | Brassicaceae | *Capsella bursa-pastoris* (L.) Medik. | shepherd's purse | AD | 10 | 7 |
| CAROL | Brassicaceae | *Cardamine oligosperma* Nutt. | little bittercress | AD | 7 | 5 |
| CERGL | Caryophyllaceae | *Cerastium glomeratum* Thuill. | sticky chickweed | AD | 3 | 0 |
| CHEAL | Chenopodiaceae | *Chenopodium album* L. | common lambsquarters | AD | 16 | 0 |
| CIRAR | Asteraceae | *Cirsium arvense* (L.) Scop. | Canada thistle | PD | 3 | 12 |
| CIRVU | Asteraceae | *Cirsium vulgare* (Savi) Ten. | bull thistle | BD | 22 | 1 |
| CONAR | Convolvulaceae | *Convolvulus arvensis* L. | field bindweed | PD | 3 | 2 |
| CYXEC | Poaceae | *Cynosurus echinatus* L. | dogtailgrass | AM | 27 | 7 |
| DAUCA | Apiaceae | *Daucus carota* L. | wild carrot | BD | 14 | 25 |
| DIGSA | Poaceae | *Digitaria sanguinalis* (L.) Scop. | crabgrass | AM | 26 | 28 |
| ECHCG | Poaceae | *Echinochloa crus-galli* (L.) P. Beauv. | barnyardgrass | AM | 18 | 25 |
| FESRU | Poaceae | *Festuca rubra* L. | red fescue | AM | 14 | 11 |
| FESSC | Poaceae | *Festuca scabrella* L. | rough fescue | PM | 6 | 0 |
| GERDI | Geraniaceae | *Geranium dissectum* L. | cut-leaf geranium | AD | 16 | 24 |
| HOLLA | Poaceae | *Holcus lanatus* L. | common velvetgrass | PM | 5 | 1 |
| HRYRA | Asteraceae | *Hypochaeris radicata* L. | common catsear | PD | 5 | 19 |
| KICEL | Scrophulariaceae | *Kickxia elatine* (L.) Dumort. | sharppoint fluvellin | AD | 5 | 18 |
| LACSE | Asteraceae | *Lactuca serriola* L. | prickly lettuce | AD | 25 | 0 |
| LAMAM | Lamiaceae | *Lamium amplexicaule* L. | henbit | AD | 0 | 4 |
| LEBNT | Asteraceae | *Leontodon nudicaulis* (L.) Banks ex Schinz & R. Keller | hairy hawkbit | BD | 6 | 4 |
| MALNE | Malvaceae | *Malva neglecta* Wallr. | common mallow | AD | 0 | 5 |
| MEDLU | Fabaceae | *Medicago lupulina* L. | black medic | AD | 5 | 6 |
| MYODI | Boraginaceae | *Myosotis discolor* Pers. | changing forget-me-not | AD | 24 | 2 |
| PANCA | Poaceae | *Panicum capillare* L. | witchgrass | AM | 21 | 1 |
| PANDI | Poaceae | *Panicum dichotomiflorum* Michx. | fall panicum | AM | 1 | 0 |
| PANMI | Poaceae | *Panicum miliaceum L.* | wild-proso millet | AM | 16 | 1 |

**Appendix A.1: (Continued)**

| Bayer code | Family | Species | Common name | Life form | Frequency[a] | |
|---|---|---|---|---|---|---|
| | | | | | Expt1 | Expt2 |
| PLALA | Plantaginaceae | *Plantago lanceolata* L. | buckhorn plantain | PD | 22 | 20 |
| PLAPR | Plantaginaceae | *Plantago patagonica* Jacq. | woolly plantain | AD | 22 | 3 |
| POACO | Poaceae | *Poa compressa* L. | Canada bluegrass | PM | 2 | 12 |
| POLAV | Polygonaceae | *Polygonum aviculare* L. | prostrate knotweed | AD | 2 | 3 |
| POROL | Portulacaceae | *Portulaca oleracea* L. | common purslane | AD | 24 | 26 |
| RUB | Rosaceae | *Rubus* spp. | wild blackberry | PD | 0 | 1 |
| RUMCR | Polygonaceae | *Rumex crispus* L. | curly dock | PD | 0 | 2 |
| SENVU | Asteraceae | *Senecio vulgaris* L. | common groundsel | AD | 6 | 2 |
| SETLU | Poaceae | *Setaria pumila* (Poir.) Roemer & J.A. Schultes | yellow foxtail | AM | 1 | 0 |
| SOLNI | Solanaceae | *Solanum nigrum* L. | black nightshade | AD | 8 | 18 |
| SOLSA | Solanaceae | *Solanum sarrachoides* Sendtner | hairy nightshade | AD | 13 | 20 |
| SONAS | Asteraceae | *Sonchus asper* (L.) Hill | prickly thistle | AD | 2 | 10 |
| TAROF | Asteraceae | *Taraxacum officinale* F. H. Wigg | common dandelion | PD | 7 | 18 |
| TRFRE | Fabaceae | *Trifolium repens* L. | white clover | PD | 10 | 11 |
| VERPE | Scrophulariaceae | *Veronica persica* Poir. | Persian speedwell | AD | 2 | 14 |
| VICSA | Fabaceae | *Vicia sativa* L. | common vetch | PD | 14 | 0 |

[a] plot frequency of 28 plots in each experiment.

**Appendix A.2:** Pearson's regression coefficient (*r*) of weed species variable to NMS axes in Experiment 1 and 2. See Appendix A.1 for weed species name lists.

| Species | Axis 1 | Axis 2 | Axis 3 | Species | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|---|---|---|---|
| | *r* | *r* | *r* | | *r* | *r* | *r* |
| | --------Expt 1------- | | | | --------Expt 2------- | | |
| POROL | -0.777 | 0.365 | -0.014 | ECHCG | -0.798 | 0.166 | 0.239 |
| ECHCG | -0.776 | -0.277 | 0.046 | ALLVI | -0.642 | -0.425 | 0.002 |
| CIRVU | -0.474 | -0.395 | 0.220 | SOLSA | -0.367 | -0.510 | -0.030 |
| TAROF | -0.416 | -0.170 | 0.177 | PLALA | -0.120 | -0.040 | -0.439 |
| SOLSA | -0.384 | -0.447 | -0.460 | CYXEC | -0.107 | 0.166 | -0.444 |
| SOLNI | -0.353 | 0.080 | -0.072 | LAMAM | -0.089 | -0.216 | -0.262 |
| VICSA | -0.348 | -0.078 | -0.133 | FESRU | 0.012 | -0.145 | -0.195 |
| DAUCA | -0.334 | -0.067 | 0.480 | POROL | 0.064 | 0.209 | 0.357 |
| DIGSA | -0.315 | -0.074 | -0.495 | GERDI | 0.086 | -0.273 | -0.333 |
| TRFRE | -0.231 | 0.182 | 0.197 | SONAS | 0.087 | -0.349 | 0.116 |
| POLAV | -0.218 | 0.640 | 0.458 | PLAPR | 0.113 | -0.200 | -0.099 |
| CIRAR | -0.212 | 0.677 | 0.446 | TRFRE | 0.145 | -0.010 | -0.343 |
| SONAS | -0.202 | 0.661 | 0.471 | VERPE | 0.167 | -0.259 | -0.482 |
| VERPE | -0.167 | 0.239 | 0.207 | DIGSA | 0.169 | -0.525 | -0.141 |
| POACO | -0.126 | 0.720 | 0.457 | CAROL | 0.175 | 0.193 | 0.170 |
| LEBNT | -0.076 | 0.651 | 0.509 | CIRAR | 0.176 | 0.007 | -0.102 |
| KICEL | -0.070 | 0.393 | 0.569 | TAROF | 0.176 | -0.123 | 0.251 |
| HOLLA | -0.061 | -0.042 | -0.339 | CAPBP | 0.177 | 0.112 | 0.238 |
| AMARE | -0.041 | -0.502 | -0.127 | POLAV | 0.232 | 0.513 | -0.605 |
| CERGL | 0.028 | 0.318 | 0.008 | DAUCA | 0.239 | -0.050 | -0.465 |
| HRYRA | 0.050 | 0.541 | 0.645 | MALNE | 0.368 | 0.123 | -0.468 |
| ALLVI | 0.114 | -0.438 | 0.050 | AMARE | 0.406 | -0.159 | -0.534 |
| FESRU | 0.161 | -0.157 | 0.093 | SENVU | 0.419 | 0.139 | 0.261 |
| MEDLU | 0.208 | -0.196 | -0.239 | KICEL | 0.441 | 0.212 | 0.451 |
| PLALA | 0.220 | 0.002 | -0.338 | LEBNT | 0.462 | 0.124 | 0.243 |
| CAROL | 0.239 | 0.295 | -0.049 | MYODI | 0.462 | -0.069 | -0.190 |
| FESSC | 0.246 | -0.331 | -0.318 | CONAR | 0.471 | 0.437 | -0.546 |
| PANMI | 0.264 | -0.042 | 0.416 | HRYRA | 0.485 | 0.123 | -0.040 |
| CAPBP | 0.267 | -0.110 | -0.392 | RUMCR | 0.515 | 0.529 | -0.460 |
| PLAPR | 0.291 | -0.090 | -0.308 | MEDLU | 0.529 | 0.442 | -0.439 |
| MYODI | 0.334 | -0.024 | 0.079 | SOLNI | 0.542 | 0.504 | -0.159 |
| SENVU | 0.337 | -0.100 | -0.096 | POACO | 0.661 | 0.507 | -0.410 |
| CONAR | 0.357 | -0.235 | -0.317 | | | | |
| CYXEC | 0.364 | -0.544 | -0.013 | | | | |
| LACSE | 0.368 | -0.360 | -0.603 | | | | |
| GERDI | 0.420 | 0.001 | 0.133 | | | | |
| CHEAL | 0.478 | -0.483 | 0.398 | | | | |
| PANCA | 0.739 | -0.241 | -0.311 | | | | |

# APPENDIX B

**Appendix B.1:** Life form, plot frequency in each experiment of plant species observed in the sample plots at Lewis-Brown Horticulture Research Farm 43º 33´ 10˝ N, 123º 12´ 53˝ W), Oregon State University, Corvallis, OR in Summer 2011. Life form codes: AD = annual dicot; AM = annual monocot; BD = biennial dicot; PD = perennial dicot; PM = perennial monocot; PF = perennial fern ally.

| Bayer Code | Family | Species | Common Name | Life form | Frequency[a] | |
|---|---|---|---|---|---|---|
| | | | | | Expt1 | Expt2 |
| ALLVI | Liliaceae | *Allium vineale* L. | wild garlic | PM | 0 | 12 |
| AMARE | Amaranthaceae | *Amaranthus retroflexus* L. | redroot pigweed | AD | 0 | 4 |
| ANGAR | Caryophyllaceae | *Spergularia rubra* (L.) J. & K. Presl | red sandspurry | AD | 0 | 0 |
| CAPBP | Brassicaceae | *Capsella bursa-pastoris* (L.) Medik. | shepherd's purse | AD | 6 | 2 |
| CAROL | Brassicaceae | *Cardamine oligosperma* Nutt. | little bittercress | AD | 0 | 3 |
| CHAAN | Onagraceae | *Chamerion angustifolium* (L.) Holub | fireweed | PD | 2 | 1 |
| CIRAR | Asteraceae | *Cirsium arvense* (L.) Scop. | Canada thistle | PD | 1 | 8 |
| CYXEC | Poaceae | *Cynosurus echinatus* L. | dogtailgrass | AM | 0 | 2 |
| DAUCA | Apiaceae | *Daucus carota* L. | wild carrot | BD | 12 | 13 |
| DIGSA | Poaceae | *Digitaria sanguinalis* (L.) Scop. | crabgrass | AM | 8 | 7 |
| ECHCF | Poaceae | *Echinochloa frumentacea* Link | Japanese millet | AM | 19 | 20 |
| ECHCG | Poaceae | *Echinochloa crus-galli* (L.) P. Beauv. | barnyardgrass | AM | 1 | 1 |
| EQUAR | Equisetaceae | *Equisetum arvense* L. | field horsetail | PF | 3 | 19 |
| EROCI | Geraniaceae | *Erodium cicutarium* (L.) L'Hér. ex Ait. | redstem filaree | AD | 19 | 19 |
| FESRU | Poaceae | *Festuca rubra* L. | red fescue | AM | 14 | 0 |
| GERDI | Geraniaceae | *Geranium dissectum* L. | cut-leaf geranium | AD | 17 | 18 |
| GRASS | Poaceae | Unidentified grass seedling | | AM | 0 | 14 |
| HRYRA | Asteraceae | *Hypochaeris radicata* L. | hairy cats-ear | PD | 1 | 6 |
| KICEL | Scrophulariaceae | *Kickxia elatine* (L.) Dumort. | sharppoint fluvellin | AD | 3 | 3 |
| LAMAM | Lamiaceae | *Lamium amplexicaule* L. | henbit | AD | 9 | 8 |
| LEBNT | Asteraceae | *Leontodon nudicaulis* (L.) Banks ex Schinz & R. Keller | hairy hawkbit | BD | 11 | 7 |
| MALNE | Malvaceae | *Malva neglecta* Wallr. | common mallow | AD | 1 | 0 |
| MEDLU | Fabaceae | *Medicago lupulina* L. | black medic | AD | 0 | 1 |
| PANCA | Poaceae | *Panicum capillare* L. | witchgrass | AM | 1 | 0 |
| PLALA | Plantaginaceae | *Plantago lanceolata* L. | buckhorn plantain | PD | 9 | 3 |
| POAAN | Poaceae | *Poa annua* L. | annual bluegrass | AM | 8 | 18 |
| POLCO | Polygonaceae | *Polygonum convolvulus* L. | wild buckwheat | AD | 0 | 2 |
| POLAV | Polygonaceae | *Polygonum aviculare* L. | prostrate knotweed | AD | 1 | 2 |
| POROL | Portulacaceae | *Portulaca oleracea* L. | common purslane | AD | 14 | 11 |
| RAPRA | Brassicaceae | *Raphanus raphanistrum* L. | wild radish | AD | 4 | 0 |
| RORUF | Brassicaceae | *Rorippa curvisiliqua* (Hook.) Bess. ex Britt. | curvepod yellowcress | AD | 0 | 5 |

**Appendix B.1: (Continued)**

| Bayer Code | Family | Species | Common Name | Life form | Frequency[a] | |
|---|---|---|---|---|---|---|
| | | | | | Expt1 | Expt2 |
| RUB | Rosaceae | *Rubus* spp. | wild blackberry | PD | 1 | 0 |
| RUMAA | Polygonaceae | *Rumex acetosella* L. | red sorrel | PD | 18 | 11 |
| SENVU | Asteraceae | *Senecio vulgaris* L. | common groundsel | AD | 6 | 9 |
| SOLNI | Solanaceae | *Solanum nigrum* L. | black nightshade | AD | 1 | 5 |
| SONAS | Asteraceae | *Sonchus asper* (L.) Hill | prickly thistle | AD | 15 | 17 |
| SPPRU | Caryophyllaceae | *Spergularia rubra* (L.) J. & K. Presl | red sand-spurry | AD | 0 | 3 |
| TAROF | Asteraceae | *Taraxacum officinale* F. H. Wigg | common dandilion | PD | 5 | 4 |
| TRFRE | Fabaceae | *Trifolium repens* L. | white clover | PD | 20 | 18 |
| VERPE | Scrophulariaceae | *Veronica persica* Poir. | Persian speedwell | AD | 14 | 14 |
| VICSA | Fabaceae | *Vicia sativa* L. | common vetch | PD | 4 | 6 |

[a] plot frequency out of 28 sampling plots in each experiment.

**Appendix B.2:** Correlation of weed species variable to NMSs for analysis of amended material on Experiment 1 and 2. See Appendix B.1 for weed species name lists.

| Species | NMS1 | NMS2 | Species | NMS1 | NMS2 |
|---|---|---|---|---|---|
| | $r$[a] | $r$ | | $r$ | $r$ |
| | ------Expt 1------ | | | ------Expt 2------ | |
| PANCA | 0.466 | -0.204 | ECHCG | 0.501 | -0.657 |
| EQUAR | 0.355 | 0.554 | POLCO | 0.176 | -0.288 |
| POLAV | 0.132 | -0.177 | ALLVI | 0.096 | 0.364 |
| VICSA | 0.112 | 0.015 | CAROL | 0.037 | -0.445 |
| HRYRA | -0.084 | 0.079 | AMARE | 0.027 | 0.291 |
| DAUCA | -0.127 | 0.420 | VICSA | -0.160 | 0.370 |
| RUB | -0.136 | -0.079 | RORCU | -0.163 | -0.499 |
| MALNE | -0.151 | 0.255 | CHAAN | -0.218 | -0.050 |
| CIRAR | -0.172 | 0.026 | KICEL | -0.226 | -0.231 |
| ECHCG | -0.211 | 0.173 | CYXEC | -0.258 | 0.150 |
| SOLNI | -0.211 | 0.173 | MEDLU | -0.269 | -0.141 |
| CHAAN | -0.219 | 0.036 | SPBRU | -0.296 | -0.186 |
| TAROF | -0.264 | 0.203 | CAPBP | -0.327 | -0.178 |
| KICEL | -0.267 | 0.240 | CIRAR | -0.353 | -0.286 |
| RAPRA | -0.272 | 0.360 | PLALA | -0.356 | -0.008 |
| DIGSA | -0.289 | 0.302 | POLAV | -0.358 | -0.055 |
| SENVU | -0.342 | 0.232 | TAROF | -0.385 | 0.124 |
| CAPBP | -0.387 | 0.188 | HRYRA | -0.389 | 0.155 |
| RUMAA | -0.472 | 0.450 | DIGSA | -0.405 | -0.277 |
| LEBNT | -0.484 | 0.158 | SOLNI | -0.407 | -0.019 |
| POAAN | -0.491 | 0.426 | ECHCF | -0.417 | 0.100 |
| EROCI | -0.494 | 0.289 | RUMAA | -0.452 | -0.081 |
| PLALA | -0.500 | 0.215 | TRFRE | -0.464 | -0.130 |
| VERPE | -0.542 | 0.281 | SENVU | -0.470 | -0.048 |
| LAMAM | -0.551 | 0.513 | LAMAM | -0.485 | 0.032 |
| TRFRE | -0.610 | 0.420 | LEBNT | -0.507 | -0.236 |
| GERDI | -0.655 | 0.117 | DAUCA | -0.607 | -0.094 |
| FESRU | -0.687 | 0.295 | VERPE | -0.636 | -0.242 |
| POROL | -0.739 | 0.313 | GERDI | -0.769 | -0.16 |
| ECHCF | -0.848 | 0.169 | POROL | -0.772 | -0.165 |
| SONAS | -0.853 | 0.355 | GRASS | -0.794 | -0.366 |
| | | | POAAN | -0.816 | -0.120 |
| | | | EROCI | -0.829 | -0.360 |
| | | | EQUAR | -0.870 | 0.281 |
| | | | SONAS | -0.911 | -0.140 |

[a] $r$, Pearson's regression coefficient.

# APPENDIX C

**Appendix C.1:** Sole carbon sources (31 substrates) in the Biolog EcoPlates™.

| C sources | | |
|---|---|---|
| Amines | Carbohydrates | Carboxylic acids |
| putrescine | α-D-lactose | α-ketoglutaric acid |
| phenylethylamine | β-methyl-D-glucoside | D-galacturonic acid |
| | D-cellobiose | D-glucosaminic acid |
| Amino acids | D-mannitol | D-malic acid |
| arginine | *i*-erythritol | itaconic acid |
| L-asparagine | glucose-1-phosphate | methyl pyruvate |
| L-phenylalanine | D-xylose | γ-hydroxybutyric acid |
| L-serine | D-galactonic acid γ-lactone | |
| L-threonine | *N*-acetyl-D-glucosamine | Polymers |
| glycyl-L-glutamic acid | D,L-α-glycerol phosphate | α-cyclodextrin |
| | | glycogen |
| | Phenolic compounds | tween 40 |
| | 2-hydroxy benzoic acid | tween 80 |
| | 4-hydroxy benzoic acid | |

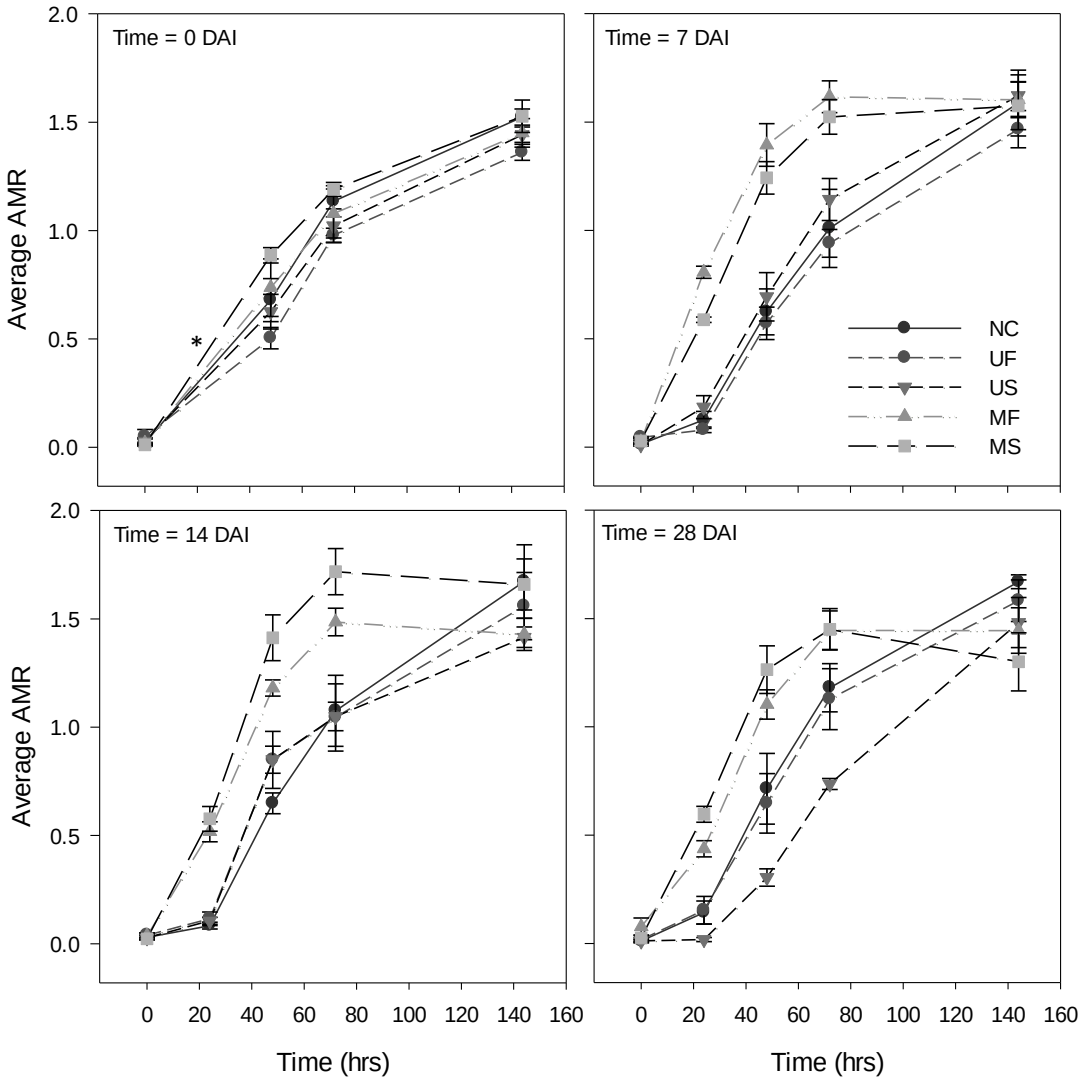
**Appendix C.2:** Total $CO_2$ of basal respiration after incubation in the dark for 48 hours at 25 ºC on different days after meal or urea incorporation (DAI).

| Expt | Trt[a] | Basal respiration[b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ----------μg $CO_2$-C $g^{-1}$ soil $hr^{-1}$---------- | | | | | | | | | | | |
| 1 | | 0 DAI | | | 7 DAI | | | 14 DAI | | | 28 DAI | | |
| | NC | 3.7 | (0.42) | fg | 2.8 | (0.30) | fg | 2.5 | (0.13) | fg | 1.4 | (0.32) | g |
| | UF | 3.8 | (1.05) | ef | 2.6 | (0.30) | fg | 2.6 | (0.16) | fg | 2.0 | (0.07) | g |
| | US | 7.2 | (2.02) | fg | 3.6 | (0.82) | fg | 2.1 | (0.29) | fg | 1.3 | (0.19) | fg |
| | MF | 376.2 | (92.37) | a | 96.9 | (6.40) | ab | 44.0 | (6.44) | bcd | 17.0 | (1.51) | de |
| | MS | 118.0 | (52.27) | bc | 68.8 | (17.43) | bcd | 63.1 | (18.42) | bcd | 25.7 | (6.73) | cde |
| 2 | | 0 DAI | | | 7 DAI | | | 14 DAI | | | 28 DAI | | |
| | NC | 4.6 | (0.31) | ghij | 2.5 | (0.20) | hijk | 1.9 | (0.26) | ijk | 1.7 | (0.04) | ijkl |
| | UF | 6.3 | (1.35) | gh | 2.7 | (0.33) | hijk | 1.8 | (0.30) | ijkl | 0.8 | (0.29) | l |
| | US | 5.2 | (1.39) | ghi | 4.0 | (1.02) | ghijk | 1.8 | (0.27) | ijkl | 1.3 | (0.06) | kl |
| | MF | 238.3 | (57.97) | ab | 82.4 | (12.96) | bcd | 37.7 | (11.30) | de | 9.8 | (1.39) | fg |
| | MS | 255.2 | (44.54) | a | 142.6 | (10.22) | abc | 66.3 | (6.90) | cd | 22.2 | (5.64) | ef |

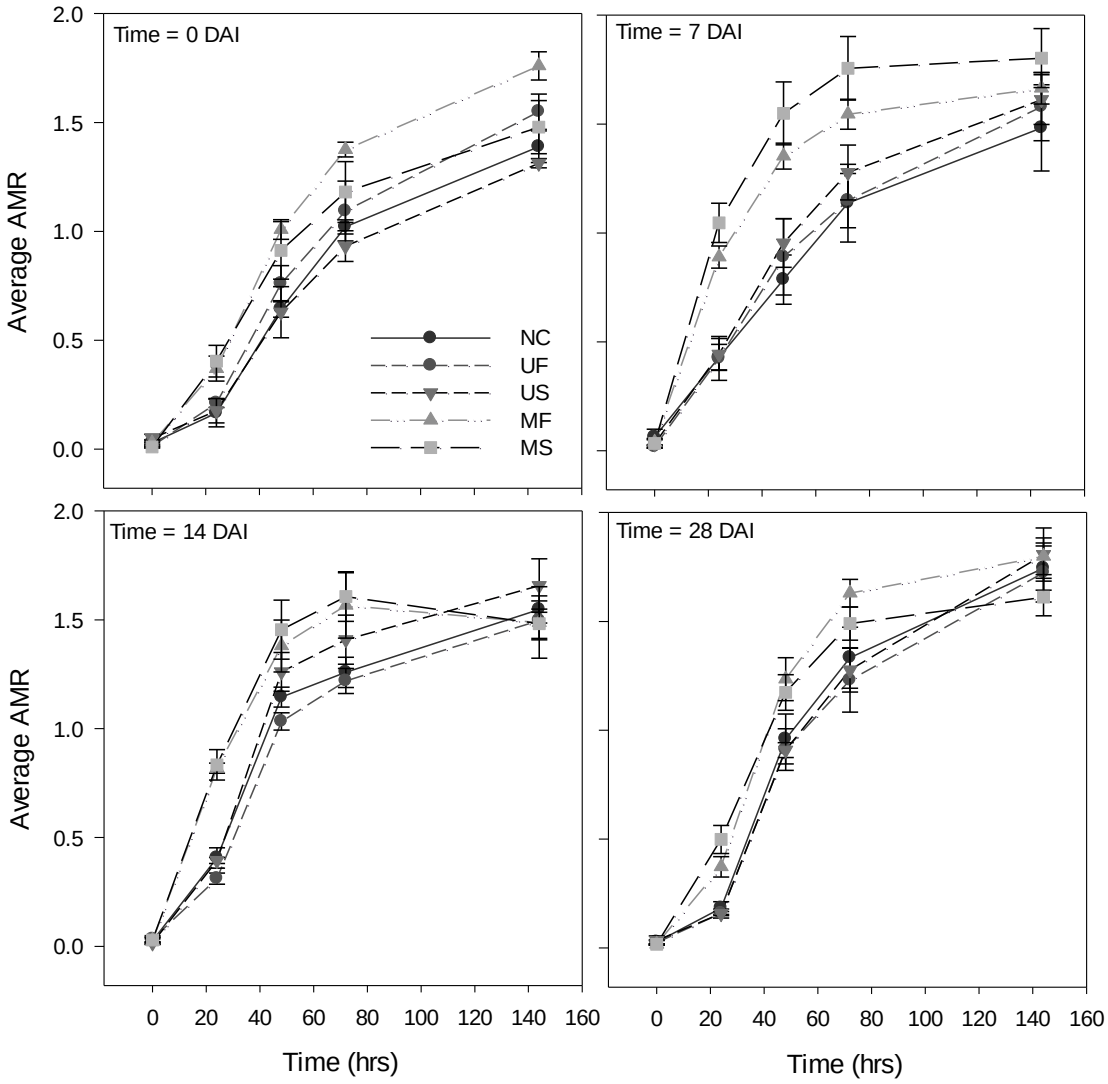
[a] Treatments: NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam amendment.

[b] Data are represented as means with SE within parentheses. Different letters within an experiment indicate significant differences at the 0.05 level using Tukey's HSD.

**Appendix C.3:** Community-level physiological profiling comparing the average metabolic response (AMR) of five soil treatments over incubation time at different sampling times in Experiment 1. * = missing data; DAI = days after initial material incorporation; NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.



**Appendix C.4:** Community-level physiological profiling comparing the average metabolic response (AMR) of five soil treatments over incubation time at different sampling times in Experiment 2. DAI = days after initial material incorporation; NC = non-amended; UF = full rate of urea amendment; US = split rate of urea amendment; MF = full rate of meadowfoam seed meal amendment; MS = split rate of meadowfoam seed meal amendment.)



**Appendix C.5:** Correlation of carbon source variable to PCs for analysis of amended materials on Experiment 1 and 2.

| PC 1 | | PC 2 | |
|---|---|---|---|
| Carbon source | $r^a$ | Carbon source | *r* |
| ----------Expt 1---------- | | | |
| Amines | | Amino acids | |
| Phenylethylamine | -0.663 | L-Phenylalanine | -0.560 |
| Polymers | | L-Serine | 0.592 |
| α-Cyclodextrin | -0.629 | Carbohydrates | |
| Phenolic compounds | | D-Xylose | 0.663 |
| 2-Hydroxy Benzoic Acid | -0.537 | Phenolic compounds | |
| Carbohydrates | | 4-Hydroxy Benzoic Acid | -0.583 |
| *i*-Erythritol | -0.563 | | |
| D-Cellobiose | 0.508 | | |
| D-Galactonic Acid γ-Lactone | 0.645 | | |
| D-Mannitol | 0.910 | | |
| *N*-Acetyl-D-glucosamine | 0.600 | | |
| Carboxylic acids | | | |
| Methyl Pyruvate | 0.538 | | |
| D-Galacturonic Acid | 0.645 | | |
| Amino acids | | | |
| L-Asparagine | 0.782 | | |
| ----------Expt 2---------- | | | |
| Amines | | Carbohydrates | |
| Phenylethylamine | -0.749 | α-D-Lactose | 0.798 |
| Polymers | | Amino acids | |
| α-Cyclodextrin | -0.678 | L-Phenylalanine | -0.687 |
| glycogen | -0.724 | | |
| Phenolic compounds | | | |
| 2-Hydroxy Benzoic Acid | -0.631 | | |
| Carbohydrates | | | |
| Glucose-1-Phosphate | 0.678 | | |
| D-Cellobiose | 0.785 | | |
| β-Methyl-D-glucoside | 0.792 | | |
| D-Xylose | 0.676 | | |
| D-Galactonic Acid γ-Lactone | 0.810 | | |
| D-Mannitol | 0.854 | | |
| *N*-Acetyl-D-Glucosamine | 0.713 | | |
| Carboxylic acids | | | |
| D-Glucosaminic acid | 0.534 | | |
| D-Galacturonic Acid | 0.637 | | |
| Amino acids | | | |
| L-Asparagine | 0.898 | | |
| L-Serine | 0.561 | | |

[a] *r*, Pearson's regression coefficient.