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The Potential of Green Manure Mixtures to Provide Nutrients to a Subsequent Lettuce Crop

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ABSTRACT

The objectives of this greenhouse experiment were to quantify the benefits of green manure (GM) diversity and to assess effect of GM on a succeeding lettuce crop. Species included barley (B), field pea (P), sunn hemp (S), and buckwheat (U). Treatments included single plantings of B and P and mixtures of B and P (BP), B, P, and S (BPS), and B, P, S, and U (BPSU). The pea GM had the highest shoot concentration of potassium, calcium, magnesium, manganese, zinc, and iron. Roots had higher concentrations of nutrients than shoots. Accumulation of nutrients in legumes was higher than in barley. The CO_2 flux from the soil suggests that the most intensive mineralization period occurred within two weeks after incorporation of GMs. The mixtures and single planting of P mixtures had a significant benefit over single planting of B regarding fresh and dry weight of the lettuce.

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Introduction

Emphasis on the maintenance of environmental quality in response to use of chemical fertilizers in crop production has increased worldwide. Conventional cropping systems require continuous applications of chemical fertilizers that are vulnerable to large losses of nutrients into the environment. Zhu et al. (2005) reported that only 10% of applied N fertilizer was recovered in above-ground biomass of pepper (*Capsicum frutescens* L.), and about 52% was lost from the plant-soil system in greenhouse experiments. Recovery of applied N in crop plants in fields is usually less than 50% (Fageria and Baligar 2005). Green manuring may be an environmentally sound alternative to chemical fertilization. In field production, a well-chosen green manure (GM) may balance nutrient supply (Delgado et al. 2001; Gaston, Boquet, and Bosch 2003), enhance soil biological activity and colonization of mycorrhizae (Nicholls and Altieri 2001), and increase root growth and organic matter content of the soil (Biederbeck et al. 1998). GMs contribute to the residual pool of organic N and P in the soil from which these nutrients are released slowly and N leaching loss and P fixation are restricted (Caswell et al. 1991). GMs improve the cation exchange capacity of soil, increase soil water retention, promote soil aggregation, and buffer the soil against changes in acidity (Calegar et al. 2013; Paul et al. 2013).

Several researchers have demonstrated the beneficial effects of GMs to mitigate the deficiency of many secondary and micronutrients in fields that continuously have received only N, P, and K fertilizers without any micronutrient or organic fertilizer (Dube, Childuza, and Muchaonyerwa 2013; Fageria et al. 2016; Nascente, Stone, and Crusciol 2015).

The potential benefits of GMs as a source of nutrients to crops can be achieved only if their decomposition and nutrient-release patterns are known so that the synchrony of nutrient release with

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crop nutrient demand can be improved (Myers et al. 1994). If the nutrient content and release rate from GM are too low or slow to meet crop requirements in a short time, nutrient deficiency may occur.

Proper assessment of GM management requires a greater understanding of the site-specific relationships between the life cycles of the plants used (GM or cash crops), environment (climate, weather, soil, and pests), and management options as well as production goals. Management options of GM include the selection of plant materials with different chemical composition (quality) as well as timing, quantity, and form of application into the soil (Palm et al. 2001). Using multispecies cover crops mixtures, called "cocktails" and consisting of three or more species as GM, can maximize soil organic matter diversity and therefore affect decomposition rate and N availability in soil. Evidently, a wide range of traits and functions in diverse combination of species is a key factor for better performance of cocktails especially under changing environmental conditions.

Winter grass or brassica species absorb and recycle N if N in excess of needs of crops occurs. Legumes in the mixture provide a N supplement for the next crop if N is needed for fertilization. The success of a cocktail depends on each species in the mix providing the appropriate balance with other species in the mix. Achieving this balance can be difficult because certain species are highly competitive, causing the desired services of the less competitive species to go unrealized.

Nonlegume cover crops, such as barley (*Hordeum vulgare* L.) and buckwheat (*Fagopyrum esculentum* Moench) increase soil organic C and recover inorganic N to restrict leaching (Kuo, Sainju, and Jellum 1997). Legume cover crops, such as sunn hemp (*Crotalaria juncea* L.) and pea (*Pisum sativum* L.) may enrich soil N and consequently enhance cash crop yields. As a result, legume cover crops can reduce the amount of N fertilization needed for succeeding crops (Kuo and Jellum 2002). Selection of species in a cocktail aims to include warm-season broadleaf species, cool-season broadleaf species, and warm-season and cool-season grasses. Different species and plant parts in GMs (i.e., leaves, stems) are likely to influence the decomposition and nutrient-release rates (Handayanto, Giller, and Cadish 1997).

Surveys indicate widespread use of GM, but it remains unclear which GM species are used, how they are used, and the type and degree of production benefit that the GM provides. Multispecies cover cropping systems have been tested in previous studies, but most research was not designed to quantify the benefits of increasing cover crop diversity. Typically, studies with crop mixtures in GMs compare monoculture species with biculture combinations of those species (Kuo and Jellum 2002). Although some focus on more diverse mixtures of cover crops has occurred, characterization of the benefits associated with increasing diversity are limited often to simple dry weight comparisons of growth. Many cover crop mixture studies fail to include monoculture control treatments necessary to evaluate the potential benefits or antagonisms of the different mixtures (Madden et al. 2004).

Green leafy vegetables often contain high nitrate levels (Prasad and Chetty 2008), and lettuce is classified as having a very high nitrate content (Santamaria 2006). Because consuming high levels of nitrate may lead to severe pathologies in human (Mensinga, Speijers, and Meulenbelt 2003), cultivating edible crops with low nitrate content is very important. Regarding lettuce, the type of N, timing of N release, light intensity, and lettuce type affect the accumulation of nitrates in this crop (Gunes and Aktas 1995; Gutierrez et al. 2002; Pavlou, Ehaliotis, and Kavvadias 2007; Tesi and Lenzi 1998). Therefore, sustainable practices are needed to maintain yield and ensure quality in lettuce production. The aim of this study was to investigate the growth of different legume and nonlegume cover crops in monoculture and in mixed culture and to assess their residual effects on a succeeding lettuce crop.

Materials and methods

Study site, soil, and experimental design

A pot experiment was established under glasshouse conditions (day: 20°C, 16 h; night: 16°C, 8 h) during autumn-winter 2014 at the University of Massachusetts, Amherst. Field soil used was a Hadley fine sandy loam (typical Udifluvent, coarse-silty, mixed, non-acid, and mesic). The soil after

Table 1. Treatments for production of green manures and for evaluation of effects of green manures on lettuce growth.

Experiment part	Stand[a]	Green manure properties	
		Target density plant m^2	Seeds per pot
I	B	300	18
	P	112	6
	BP	125	9:3
	BPS	350	6:2:3
	BPSU	300	4:1:2:6
II	Lettuce (L)[b]	1 plant/pot	10

[a]Species: barley (B), pea (P), sunn hemp (S), and buckwheat (U).
[b]Lettuce was grown in pots with the additions of the green manures produced in Part I or with no green manure.

sieving (2-mm) was packed into pots (216 mm diameter × 203 mm high) to an average bulk density of 1.23 mg m^{-3} by dropping a 2.5-kg weight from a height of 350 mm five times on successive equal volume layers of soil. All pots were filled to 10 mm of the top, and soil water was maintained by weighing individual pots and replacing lost water during plant growth at two-day intervals.

Part I. Production of green manures

The experimental design for cover cropping treatments was a randomized, complete block with five plant stands (species or mixtures) of GMs and five replicates (Table 1). Stands included barley (*Hordeum vulgare* L.) (B), field pea (*Pisum sativum* L.) (P), sunn hemp (*Crotalaria juncea* L.) (S), and buckwheat (*Fagopyrum esculentum* Moench) (U). Treatments included single plantings of B and P and mixtures of B and P (BP), B, P, and S (BPS), and B, P, S, and U (BPSU). A treatment of no GM (CTRL) was included in a second experiment that evaluated growth and nutrient accumulation in lettuce. Due to discrepancy in size specific to sunn hemp, medium-sized seeds were planted with a uniform pattern in single planting and mixtures. Prior to planting, sunn hemp and field pea were inoculated with a cowpea-type rhizobium species (Johnny's Selected Seeds, Albion, Maine).

The aboveground plant parts were cut at the soil surface after 50 days. At this time, U was at the early stages of flowering, whereas B, P, and S were at the ending of their vegetative stages. Shoots of species were separated in the mixtures and weighed. Roots were separated from surrounding soil by washing through sieves with mesh size of 0.1 mm to remove soil. Roots were not separated by species. Above- and belowground dry biomass (oven dried at 65 °C), and their nutrient contents were measured for each pot. Procedures for analyses are reported below in this section. The total nutrient accumulation in GMs is reported as nutrient concentration x dry biomass of shoots or roots.

Part II. Green manure impacts on lettuce

Fresh aboveground GM parts were cut into 10-mm lengths. All materials from each pot including associated roots of GMs were incorporated into the soil for each plant stand. After 15 days during which CO_2 release was measured, 10 seeds of lettuce (*Lactuca sativa* L. cv.) were planted in pots with no GM or with GM (Table 1). Lettuce was thinned to one plant per pot after 10 days. Soil water was kept constant by weighing the pots and adding water on every third day to return to the original weight until lettuce harvest. Lettuce was harvested after 57 days and analyzed to determine marketability and nutrient content. Lack of marketability was determined based on the visibly yellow (chlorotic) and wilted leaves; green, healthy leaves were considered as marketable (McCabe et al. 2001).

Tissue analysis for nutrients

Lettuce leaf tissues were ground (1-mm) after drying, and 0.5 g of ground sample was put in a porcelain crucible and placed into muffle furnace at 500°C for 8 hours. After ignition and cooling, 15 mL of 1 M HCl were added to each crucible to dissolve the ash. The sample was filtered through paper (Whatman #2) into glass scintillation vials. Then 1 mL of solution was transferred into a 25-mL volumetric flask and filled to volume with 1 M HCl. All measurements were performed by nitrogen gas plasma spectrometry (Agilent 4200 MP-AES, Agilent Technologies, 5301 Stevens Creek Blvd. Santa Clara, CA 95051 United States). Total nitrogen was measured by a Kjeldahl procedure (Bremner and Mulvaney, 1982).

CO_2 respiration test

The first reading was made two days after GM incorporation and then every other day until planting of lettuce seeds. Forty gram of soil from each pot were added to a 250-mL jar. The bottom of the jar was tapped occasionally during filling to eliminate voids. Gel pads (Solvita & Woods Ends Laboratories, City, Maine, United States) were inserted into the soil, and the lid was screwed tightly. Jars were kept at room temperature of 21 C and from sunlight. After 24 hours, pads were removed and read with a digital color reader for analysis (Solvita & Woods Ends Laboratories, United States).

Data processing

Data were processed by analysis of variance (SAS, 9.1.3, The SAS Inst., Cary, NC; Steel and Torrie 1980). If the effect of treatment was significant ($p \leq 0.05$) by F-test, means were separated by Duncan's New Multiple Range Test (Steel and Torrie 1980).

Results

Green manure experiment

The highest shoot fresh weight biomass was produced in the combination of BPSU or with the monoculture of barley, and the lowest shoot fresh biomass was produced in the pea monoculture, the BP combination, or the BPS combination (Table 2). Dry biomass of shoots followed a trend similar to the fresh biomass. Barley or the BP combination had the highest root fresh or dry weight, and pea produced the lowest root biomass. However, the highest shoot/root weight ratio was produced in single planting of pea or in BPSU. In the mixtures of BPS or BPSU, sunn hemp had the lowest dry mass growth whereas barley or buckwheat gave the highest dry biomass (Table 3). The BPSU combination significantly increased the GM dry biomass compared with all other GMs (Tables 2 and 3).

Substantial partitioning of biomass to the roots indicates the importance of roots in a GM-vegetable cropping system. The N in roots of monoculture of pea is important in supplying N through decay since roots of pea contain the highest N concentration compared to other GM components except for sunn hemp (Table 3). The total N content of barley shoot biomass was higher in various mixtures relative to its monoculture, a property that signals an increase to the value of mixed GMs (Hwang et al. 2015). GM legumes had higher N concentrations in their shoot tissues than nonlegumes with pea or sunn hemp being high in the BP, BPS, or BPSU combination. However, the presence of pea in mixtures was a major factor to increase in final N content because of its high N concentration and biomass.

Table 2. Biomass and shoot/root mass ratios of green manure crops grown in pots in a greenhouse.

Green manure[a]	Biomass, g/pot[b]				Shoot/root ratio[b]	
	Fresh wt		Dry wt		Fresh wt	Dry wt
	Shoots	Roots	Shoots	Roots		
B	108[a]	99[a]	20.2[b]	15.2[a]	1.09[b]	1.32[c]
P	87[b]	48[c]	16.8[c]	7.3[b]	1.80[a]	2.30[a]
BP	83[b]	89[a]	17.5[bc]	15.1[a]	0.93[b]	1.16[c]
BPS	77[b]	68[b]	15.1[c]	9.1[b]	1.14[b]	1.65[bc]
BPSU	118[a]	69[b]	25.5[a]	9.8[b]	1.71[a]	2.58[ab]

[a]B, barley; P, pea; BP, barley-pea; BPS, barley-pea-sunn hemp; BPSU, barley-pea-sunn hemp-buckwheat.
[b]In columns, means followed by different letters are significantly different by Duncan's New Multiple Range Test, $P = 0.05$.

Table 3. Dry biomass production and nitrogen concentration and content in individual green manures and mixtures of green manures.

Green manure [a]	Species	Dry wt shoots, g	Dry wt roots, g[b]	Nitrogen concentration, % dry wt		Nitrogen content, mg/pot	
				Shoots	Roots[b]	Shoots	Roots[b]
B	B	20.15[b]	15.21[a]	1.47[b]	0.77[b]	290[b]	120[b]
P	P	16.78[c]	7.27[b]	2.28[a]	1.84[a]	380[a]	130[a]
BP	BP[c]	17.54[bc]	15.09[a]	1.68	0.99[b]	290[b]	130[a]
	B	9.69		1.59		150	
	P	7.85		1.79		140	
BPS	BPS[c]	15.14[c]	9.13[b]	2.00[b]	1.38[ab]	300[b]	120[b]
	B	7.23		1.51		110	
	P	5.11		2.25		115	
	S	2.80		2.78		75	
BPSU	BPSU[c]	25.46[a]	9.84[b]	1.45[b]	0.92[b]	370[a]	130[a]
	B	6.21		1.53		95	
	P	3.52		2.45		85	
	S	2.13		2.69		60	
	U	13.6		0.96		130	

[a]B, barley; P, pea; BP, barley-pea; BPS, barley-pea-sunn hemp; BPSU, barley-pea-sunn hemp-buckwheat.
[b]In columns, means of green manure treatments followed by different letters are significantly different by Duncan's New Multiple Range Test, $P = 0.05$.
[c]The concentrations and contents of N in the species of the mixtures are calculated from the concentrations of N and the weights of the components of BP, BPS, and BPSU.

GMs showed significant differences regarding phosphorus, potassium, calcium, and magnesium concentrations and contents (Tables 4 and 5). Phosphorus shoot concentration and content appeared highest in BPSU (Table 4). Alone or in mixtures, pea had the highest K concentration in shoot or root (Table 4); however, the highest shoot content of K was with BPSU combination, and the highest root content of K was with barley and was due to its dry weight (Table 5). The shoot Ca concentration was high in pea in combinations as well as its single planting (Table 4); however, shoot Ca content was the highest in BPSU combination (Table 5). Calcium concentration in root was highest in pea (Table 4); however, Ca content was the highest in the single planting of barley (Table 5). Magnesium shoot concentration and content were high in pea and in all combinations whereas barley alone had the lowest Mg concentration (Table 4). The highest root Mg concentration was in single planting of pea (Table 4); however, pea had a low root Mg content (Table 5).

Pea had the highest Mn shoot concentration in all stands. Manganese concentration in root was highest in single planting of pea (Table 4); however, because of its small biomass, it was among the lowest in root Mn content (Table 5). Zinc shoot or root concentration was significantly different between legumes and grasses with pea having the higher shoot or root concentration (Table 4). BPSU had the highest shoot Zn but the lowest root Zn content. Unlike the other elements in which nutrient concentration was high in the shoot tissue, Fe and Cu showed the opposite trend, and they were significantly higher in the root tissue. Pea or sunn hemp had significantly higher shoot Fe concentration than the other GMs; however, Fe varied in different combinations. Pea had the highest root Fe concentration; however, BPSU contained the highest shoot Fe content. This trend was not consistent in the root and BPSU had the lowest root Fe content. Copper shoot concentration was not significantly different among various combinations; however, Cu content was significantly different in shoot and root tissues with BPSU providing the highest Cu shoot content. Pea roots showed the highest Cu concentration but the lowest Cu content (Tables 4 & 5).

CO_2 respiration test

The line graph shows the differences in respiration measured as CO_2 release among the GM treatments (Figure 1). The treatment without a cover crop demonstrated a very low rate of respiration. All monoculture and mixed cover crops showed a high rate of early decomposition as noted by CO_2 release, which was about 58 μL CO_2 L^{-1} followed by a nearly level or a declining trend.

Table 4. Green manure shoot and root macronutrient and micronutrient concentration of crops grown in a greenhouse.

			Macronutrients, % dry wt				Micronutrients, mg/kg dry wt			
Plant part	Green manure		P	K	Ca	Mg	Mn	Zn	Fe	Cu
Shoot	B		0.23^{c}	4.09^{b}	0.96^{e}	0.25^{c}	38^{d}	24^{b}	139^{b}	10^{a}
	P		0.23^{c}	4.51^{a}	1.89^{a}	0.39^{ab}	94^{b}	31^{a}	140^{b}	11^{a}
	BP[a]		0.24	4.11	1.60	0.33	65	25	140	11
		B	0.23$_{c}$	3.78^{c}	1.03^{e}	0.28^{c}	40^{d}	21^{b}	139^{b}	11^{a}
		P	0.25^{b}	4.52^{a}	1.71^{b}	0.40^{a}	101^{b}	31^{a}	141^{b}	11^{a}
	BPS[a]		0.24	4.30	1.36	0.31	61	26	144	11
		B	0.24^{c}	4.01^{b}	1.05^{e}	0.24^{c}	42^{d}	22^{b}	136^{b}	11^{a}
		P	0.26^{b}	4.84^{a}	1.58^{b}	0.35^{b}	84^{bc}	30^{a}	153^{a}	11^{a}
		S	0.23^{c}	4.08^{b}	1.79^{a}	0.44^{a}	72^{c}	30^{a}	151^{a}	11^{a}
	BPSU[a]		0.29	3.92	1.45	0.39	88	43	127	11
		B	0.25^{b}	3.97^{b}	1.19^{d}	0.25^{c}	52^{d}	20^{b}	105^{c}	11^{a}
		P	0.30^{a}	4.41^{a}	1.92^{a}	0.41^{a}	120^{a}	32^{a}	137^{b}	11^{a}
		S	0.23^{c}	4.05^{b}	1.64^{b}	0.38^{ab}	79^{c}	31^{a}	149^{a}	11^{a}
		U	0.30^{a}	3.76^{c}	1.41^{c}	0.45^{a}	97^{b}	31^{a}	132^{b}	11^{a}
Root[b]	B		0.24^{b}	0.40^{a}	0.89^{b}	0.20^{b}	85^{b}	14^{c}	210^{b}	22^{a}
	P		0.25^{a}	0.42^{a}	1.26^{a}	0.24^{a}	102^{a}	38^{a}	284^{a}	23^{a}
	BP		0.24^{b}	0.36^{b}	0.76^{c}	0.19^{b}	90^{b}	25^{b}	236^{ab}	21^{b}
	BPS		0.25^{a}	0.41^{a}	0.69^{c}	0.18^{b}	72^{c}	20^{b}	266^{a}	21^{b}
	BPSU		0.25^{a}	0.34^{b}	0.98^{b}	0.20^{b}	76^{c}	13^{c}	186^{b}	21^{b}

[a]Nutrient concentrations in shoots of green manure mixtures are calculated from the concentrations and weights of the individual components of BP, BPS, and BPSU and are not included in the separations of means.
[b]Roots were not separated by species.
Means followed by different letters in columns are significantly different by Duncan's New Multiple Range Test, P = 0.05.

Table 5. Green manure shoot and root macronutrient and micronutrient content of crops grown in a greenhouse.

		Macronutrients, mg/pot[a]				Micronutrients, mg/pot[a]			
	Mixtures	P	K	Ca	Mg	Mn	Zn	Fe	Cu
Shoot	B	46^{b}	824^{b}	193^{b}	50^{c}	0.77^{d}	0.50^{b}	2.81^{b}	0.22^{b}
	P	38^{c}	757^{c}	317^{a}	65^{b}	1.58^{b}	0.52^{b}	2.35^{c}	0.19^{c}
	BP	42^{b}	721^{c}	234^{b}	59^{bc}	1.18^{b}	0.45^{c}	2.46^{c}	0.20^{bc}
	BPS	37^{c}	651^{d}	207^{d}	48^{c}	0.94^{c}	0.40^{d}	2.19^{d}	0.17^{d}
	BPSU	73^{a}	999^{a}	368^{a}	99^{a}	2.23^{a}	0.73^{a}	3.26^{a}	0.28^{a}
Root	B	36^{a}	61^{a}	135^{a}	30^{a}	1.29^{b}	0.221^{b}	3.20^{b}	0.34^{a}
	P	18^{c}	30^{c}	92^{b}	17^{b}	0.74^{c}	0.28^{b}	2.06^{d}	0.17^{c}
	BP	39^{a}	58^{b}	122^{a}	30^{a}	1.44^{a}	0.41^{a}	3.80^{a}	0.34^{a}
	BPS	23^{b}	37^{c}	63^{c}	16^{b}	0.66^{c}	0.18^{c}	2.43^{c}	0.19^{b}
	BPSU	25^{b}	33^{c}	96^{c}	20^{b}	0.75^{c}	1.31^{c}	1.84^{d}	0.21^{b}

[a]Means followed by different letters in columns are significantly different by Duncan's New Multiple Range Test, P = 0.05.

Pea initially showed a high rate of CO_2 release, which then declined to a release of 33 µL CO_2 L^{-1} in the ambient air of the receptacle.

Lettuce experiment

Growth

The highest number of marketable leaves per head was with single planting of pea or with the mixtures with pea and was about 40% higher than the treatment without GM (Table 6). The number of unmarketable leaves did not differ among treatments. On average, lettuce grown with GMs had

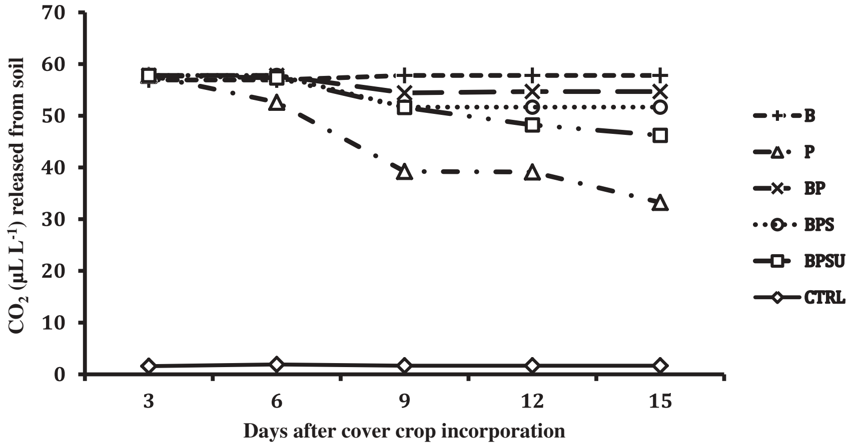

Figure 1. Carbon dioxide accumulation into ambient air of receptacle following incorporation of cover crops into soil in a greenhouse experiment. B, barley; U, buckwheat; P, pea; S, sunn hemp.

Table 6. Biomass of lettuce biomass grown in a greenhouse following green manure incorporation.

	Number of leaves per plant			Fresh wt (g/pot)			Dry wt (g/pot)		
Green manure[a]	M[b]	UM[b]	T[b]	M	UM	T	M	UM	T
B	15.3[b]	7.5[a]	22.8[b]	42.0[b]	18.8[a]	60.8[b]	4.07[b]	1.52[a]	5.59[b]
P	17.3[a]	7.4[a]	24.7[a]	57.4[a]	18.9[a]	76.3[a]	5.30[a]	1.66[a]	6.96[a]
BP	17.2[a]	7.1[a]	24.3[a]	57.2[a]	19.0[a]	76.2[a]	5.28[a]	1.73[a]	7.01[a]
BPS	17.3[a]	7.5[a]	24.8[a]	56.4[a]	19.0[a]	75.4[a]	5.23[a]	1.51[a]	6.74[a]
BPSU	17.3[a]	7.4[a]	24.7[a]	57.8[a]	19.1[a]	76.9[a]	5.48[a]	1.69[a]	7.17[a]
CTRL	11.7[c]	7.5[a]	19.2[c]	31.0[c]	12.2[b]	43.2[c]	3.19[c]	1.03[b]	4.22[c]

[a]B, barley; P, pea; BP, barley-pea; BPS, barley-pea-sunn hemp; BPSU, barley-pea-sunn hemp-buckwheat.
[b]M, marketable leaves; UM, unmarketable leaves; T, total leaves.
[c]In columns, means followed by different letters are significantly different by Duncan's New Multiple Range Test, $P = 0.05$.

about 80% higher marketable leaf fresh or dry weight than the treatment of no GM. However, the growth was not as good in the sole barley treatment as with pea or the mixtures but was better than with no GM.

Nutrient accumulation

Nitrogen concentrations in lettuce differed among GM treatments (Table 7). Among the cover crops, pea gave the highest N in marketable leaves, and barley gave the lowest. The treatment of no cover crop (CTRL) gave lower N concentrations than any cover crop treatment.

Phosphorus accumulation in lettuce differed among GMs with the highest occurring with BPSU incorporation, and the lowest concentrations occurring with the treatment of no cover crop (Table 7). Lettuce with any GM treatment had higher phosphorus concentration than the treatment with no GM. Marketable and unmarketable leaves did not appear to differ in phosphorus concentrations. Potassium concentration in lettuce leaves did not differ substantially among the GM treatments but was significantly lower with no GM than for the GM treatments for marketable or unmarketable leaves. GMs produced lettuce with higher Ca or Mg concentration compared with the treatment of no GM, but Ca or Mg in marketable leaves did not differ among GMs. Calcium concentration across all GMs was about twice as high in unmarketable leaves as in marketable leaves as unmarketable leaves were the oldest on the plant.

Table 7. Elemental concentrations in leaves of lettuce grown in pot following green manure incorporation.

		Macronutrients, % dry wt[b]					Micronutrients, mg/kg dry wt[b]			
	Green manure[a]	N	P	K	Ca	Mg	Mn	Zn	Fe	Cu
M[c]	B	1.66[cd]	0.16[cd]	0.30[f]	0.50[f]	0.50[e]	51[b]	27[b]	130[b]	11[a]
	P	2.55[a]	0.19[c]	0.36[d]	0.50[f]	0.50[e]	55[b]	31[a]	138[b]	12[a]
	BP	1.77[c]	0.19[c]	0.34[e]	0.50[f]	0.50[e]	53[b]	30[a]	135[b]	12[a]
	BPS	1.68[cd]	0.19[b]	0.35[de]	0.50[f]	0.50[e]	52[b]	27[b]	138[b]	12[a]
	BPSU	1.67[cd]	0.23[a]	0.37[d]	0.50[f]	0.50[e]	55[b]	27[b]	133[b]	12[a]
	CTRL	1.12[f]	0.10[d]	0.16[h]	0.20[g]	0.44[f]	43[c]	22[b]	137[b]	11[a]
UM[c]	B	1.33[e]	0.20[b]	0.51[b]	0.81[c]	0.57[d]	80[a]	13[d]	171[a]	11[a]
	P	2.13[b]	0.20[b]	0.54[a]	0.83[a]	0.66[a]	86[a]	14[d]	176[a]	11[a]
	BP	1.55[d]	0.20[b]	0.43[c]	0.78[d]	0.59[c]	86[a]	18[c]	166[a]	12[a]
	BPS	1.73[c]	0.20[b]	0.53[ab]	0.80[b]	0.59[c]	83[a]	15[cd]	174[a]	12[a]
	BPSU	1.38[e]	0.20[b]	0.53[a]	0.84[a]	0.64[b]	81[a]	16[c]	167[a]	11[a]
	CTRL	1.05[f]	0.10[c]	0.15[h]	0.62[e]	0.57[c]	83[a]	11[e]	167[a]	11[a]

[a]B, barley; P, pea; BP, barley-pea; BPS, barley-pea-sunn hemp; BPSU, barley-pea-sunn hemp-buckwheat; CTRL, control treatment.
[b]In columns, means followed by different letters are significantly different by Duncan's New Multiple Range Test, $P = 0.05$.
[c]M,marketable; UM, unmarketable leaves).

Discussion

A nutrient management system is one of the key factors to enhance crop production without endangering the environment. Good management often is characterized by reduced input of chemical fertilizers or by their replacement with organic materials such as GMs. Among the treatments of GMs, a single planting of pea had the highest shoot concentration of K, Ca, Mg, Mn, Zn, and Fe. Accumulation of mineral nutrients in pea or sunn hemp was higher than in barley. Legumes tend to contain more Ca than grasses; however, the concentration of K and P might be slightly higher or similar in grasses (Jukenvicius and Sabiene 2007; McDowell and Valle 2000). Overall, pea or sunn hemp accumulated more nutrients except for P in which buckwheat had the highest P accumulation. Buckwheat is characterized as a P-scavenger cover crop due to ability of its roots to acidify the rhizosphere mildly and therefore to release nutrients from the soil (Zhu, He, and Smith 2002). The nutrient composition of grasses and legumes can be affected by other factors such as environmental condition, stage of growth, species, and soil fertility. A mixture of legumes and grass often is recommended to yield a balance of mineral elements in a GM crop. In this study, the mixtures (BP, BPS, and BPSU) the produced high lettuce weights, but this production was equaled by the single GM of pea. It is apparent that pea contributed to the productivity associated with the mixtures. Overall, the addition of any GM resulted in higher biomass of lettuce than no cover crop (CTRL) or barley.

A single GM of pea had the highest impact on the N accumulation by lettuce, a value was more than twice the CTRL and about 50% greater than the accumulation with the other GMs. This effect can be attributed to a high concentration N in pea and a quick nitrogen mineralization in the legume residue. Previous studies have shown that GMs are decomposed rapidly during the first few weeks after incorporation, an action that results in significant N mineralization (Kuzyakov and Xingliang 2013; Puget and Drinkwater 2001). Shoot residue is broken down rapidly and serves as a source of N for a subsequent crop, whereas root litter decomposes more slowly and affects the short-term soil structural improvements associated with the use of GMs (Puget and Drinkwater 2001). The CO_2 flux from the soil suggests that the most intensive mineralization period occurred within two weeks after incorporation of GMs. Tosti et al. (2012) reported similar results.

Grasses may produce more GM biomass than legumes under adequate fertility levels; however, crops planted soon after large amounts of grass herbage have been incorporated into the soil may require additional nitrogen fertilizer to prevent nitrogen immobilization. Barley is usually lower in N concentration than pea; therefore, barley decomposes more slowly. This drawback is compensated in the mixtures since they provided a gradual release of N after incorporation into the soil. In this study, as noted by the sustained release of carbon dioxide from the barley with time after incorporation, the residue of the mix BPSU or pea alone decomposed faster than the single planting of barley, thus limiting the risk of N immobilization by BPSU or pea. This effect is due partly to lower C:N ratio of pea and sunn hemp and partly due to the succulence of buckwheat vegetation, which results in a rapid decomposition (Creamer and Baldwin 1999)

GM might be considered as a source of micronutrients. However, in this study only Mn accumulation was higher in marketable leaves with green manuring than with CTRL treatment. Zinc was higher in unmarketable leaves grown with GM that with CRTL. Other concentrations of other micronutrients in leaves generally were unaffected by GM treatments. Studies have shown that the quality of a GM stand (C:N ratio and micronutrient content) can be a determining factor to supply micronutrient and increase crop yields (Mishra et al. 2006). Litter decomposition has been suggested to favor the formation of soluble organo-metallic complexes in soils and to facilitate transport of micronutrients to plant root surfaces via diffusion (Barber 1995; Soltani et al. 2013).

Conclusion

A single species planting of GM may have various drawbacks such as incompatible release of minerals to correspond with plant nutrient uptake. However, synchrony of nutrient release can be enhanced by selecting a proper mixture of GM. Mixtures of GM have rapid release CO_2 during the initial 12 days after incorporation, an action that can be attributed to high microbial activity during decomposition. The higher N concentration in lettuce after incorporation of single planting of pea likely was due to lower C to N ratio in pea and rapid mineralization.

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