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ORIGINAL ARTICLE

Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: effect of planting and termination timing

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Abstract Crop-fallow systems dominate many semi-arid agricultural regions despite fallow's negative effects on soil and water quality. Annual legumes grown as a fallow-replacement crop, and terminated prior to maturity, can reduce these negative effects without substantially decreasing plant available water for the subsequent crop. Interest in growing legume green manures (LGMs) in synthetically-fertilized systems is increasing in the northern Great Plains of North America, partly due to the N-fixing capabilities of legumes; however, little is known about the effects of planting and termination time on N fixation amounts in the region. A 2-year field study was initiated in southwest Montana to determine the effects of planting time (spring or summer) and termination time (e.g. flower or pod) on the amount of N fixed by field pea (*Pisum sativum* cv. Arvika) and lentil (*Lens culinaris* cv. Richlea). Two methods, ^{15}N natural abundance and N difference, were used to quantify N fixation, with wheat or in-crop weeds as reference plants. In 2009, N fixed by spring-planted lentil was higher by pod than flower ($P = 0.03$). Termination time did not affect the amount of N fixed by spring-planted pea, despite more biomass by pod than flower. In 2010, both spring-planted crops fixed more N by pod than flower ($P < 0.01$) and more N was fixed by spring-planted than summer-planted crops ($P < 0.01$). These results should prove useful to growers interested in selecting management practices that optimize N fixation of LGMs.

Keywords *P. sativum* · *L. culinaris* · Nitrogen difference method · ^{15}N natural abundance method · Annual legume · Northern Great Plains · Cover crop

Abbreviations

LGM	Legume green manure
ND	Nitrogen difference
NA	^{15}N natural abundance

Introduction

For semi-arid dryland cropping systems of the northern Great Plains region of North America, water and nitrogen (N) are often the most limiting factors for crop growth. Crop-fallow systems continue to dominate portions of the region to conserve soil water for alternate year crop production (Padbury et al. 2002). For example, there were 1.37 million hectares of

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fallow in Montana in 2007 (Tanaka et al. 2010). Although fallow has reduced some of the agronomic risk introduced by highly variable precipitation patterns that characterize the northern Great Plains, it is one of biggest challenges to sustainable crop production in the region because of its effects on soil and water quality (Brandt 1996; Larney et al. 1994; Zentner et al. 2001). Additionally, recent volatility in fertilizer prices has increased interest in reducing off-farm N inputs. In response to these concerns, a short-season legume green manure (LGM) crop has been used as a viable alternative to full fallow periods, primarily due to legumes' N fixation capability (Pikul et al. 1997; Zentner et al. 1996).

The addition of biologically fixed N and biomass through LGMs can have a number of ecological and agronomic benefits, including an increase in soil organic matter, disruption of pest cycles, and an increase in N availability for subsequent crops (Kirkegaard et al. 2008). In turn, LGMs can provide a partial offset of fertilizer N and a reduction in the energy intensity of the system through lower N fertilizer inputs (Pimentel et al. 2005; Zentner et al. 2011).

Decisions regarding the management of LGMs in semi-arid agroecosystems are influenced by strategies that best use and conserve soil water. These decisions include selection of legume species, planting time, and termination time. In general, annual legumes such as pea and lentil provide a better tradeoff between water use and N contribution than perennial legumes (Biederbeck et al. 1996). Miller et al. (2011) showed a soil water advantage for winter pea over spring pea because winter pea matures earlier, allowing for an earlier termination date and less crop water use. However, fall-planted crops may be more susceptible to poor germination and weather-induced crop loss than spring varieties (Chen et al. 2006). A novel practice proposed for reducing inputs in reduced tillage LGM systems is to 'summer-plant' an LGM crop and allow the crop to terminate naturally (e.g., frost or drought) in the fall. Research on summer-planted LGMs in dryland cropping systems is scant, and its effects on N fixation and water use are relatively unknown. Termination timing decisions of LGMs require a compromise between maximizing N fixation and using stored soil water. Peak N fixation by legume crops has been observed to occur in the period from flower to early pod development (Jensen 1987); however, this growth period often corresponds with peak crop water use and subsequent extraction of stored soil water (Bremer et al. 1988). Terminating peas at early flower stage in Montana increased yield compared to pod stage because of differences in water use (Izard 2007; Miller et al. 2006). Nonetheless, there are situations (e.g., years of high precipitation) when extending the growth period could be advantageous to maximize N fixation amounts.

To fully assess the potential benefit of LGMs, quantifying the amount of N fixed by the LGM crop is required. A number of studies have measured N fixation by legume crops grown to maturity in the northern Great Plains (Bremer et al. 1988; Gan et al. 2010; Rennie 1984; Rennie and Dubetz 1986), yet to our knowledge, few studies have measured N fixation by LGMs terminated prior to full maturity. The primary objective of this study was to assess the effect of planting time and termination time on biomass production and N fixation by field pea and lentil. A secondary objective was to quantify and compare N fixation amounts by two distinct methods and assess their suitability for measuring N fixation in a semi-arid cropping system.

Materials and methods

Field description and experimental design

A 2-year plot study was conducted in 2009 and 2010 at two sites (1.4 km apart) on the same conventionally managed farm near Amsterdam, MT, USA (45°45′ N, 111°23′ W). Each site was established on barley (2009) or wheat (2010) stubble with at least 3 year of previous no-till management. Soil types were Amsterdam silt loam (frigid Typic Haplustoll) and Brocko silt loam (frigid Aridic Calciustept) for 2009 and 2010, respectively. Soil properties for both sites are summarized in Table 1. Growing season precipitation was measured at each site with a tipping bucket rain gauge (Hobo, Onset Corp., Bourne, MA, USA). Over-winter precipitation, monthly mean high temperature averages, and long-term average data were gathered from the nearest meteorological station located 19 km from the site in Belgrade, MT, USA.

The study was a randomized complete block design consisting of four blocks. Plots were 8 by 12 m. Main plot factors were (1) legume species (pea or lentil); (2) planting time; and (3) termination time. Planting times were winter, spring, or summer; however, winter-planted plots failed to fully establish in both years and

Table 1 General soil properties for 2009 and 2010 study sites measured at spring seeding

Soil property	2009	2010
Soil texture	Clay loam/loam	Silt loam
Soil pH[a]	6.6–8.0	7.9–8.4
Organic carbon (g kg^{-1})	15	14
Olsen P (mg kg^{-1})	30	18
K (mg kg^{-1})[b]	486	561
SO_4-S (mg kg^{-1})	8	11
Spring NO_3–N (kg N ha^{-1})[c]	29	46
Summer NO_3–N (kg N ha^{-1})[c]	28	33

Soil characteristics are either the range or mean for 12 samples collected from the 0–0.15 m depth, unless otherwise stated. Each sample was a composite of four subsamples per location

[a] 1:1 mixture of soil:water

[b] Ammonium acetate K

[c] Averaged across all planted plots to a 0.9-m depth

were not included in this study. Termination timings were flower (50% of plants had one open flower), intermediate (7 days after flower; 2009 spring-planting only), pod (50% of plants had one flat-pod), or natural crop senescence due to drought or frost, whichever came first (2009 summer-planting only). Drought-induced senescence and grasshopper herbivory of natural-terminated crops in 2009 resulted in a large loss of plant tissue N and data were not reported. Based on this observation, the natural termination was replaced with pod termination in 2010. In both years, a 2-m strip of spring wheat (*Triticum aestivum* cv. Choteau) was planted adjacent to each legume plot to serve as a non-N-fixing reference plant.

Crop management, field, and laboratory measurements

Agronomic management factors for both years are given in Table 2. Fungicide-treated seed was sown directly into stubble with a seed row spacing of 0.25 m (2009) and 0.30 m (2010). Commercial granular rhizobia inoculum (Optimize Pulse IF, EMD Crop Bioscience, Brookfield, WI, USA) was applied at 2.8 kg $ha^{-1}$with the legume seeds. At each seeding, monoammonium phosphate fertilizer was midrow banded at an equivalent N rate of 6 kg ha^{-1} for legume plots. Added N fertilizer levels were small compared to soil NO_3–N concentrations at time of seeding and were not expected to substantially affect legume N fixation. All crops, with the exception of natural, were terminated with glyphosate sprayed at a rate of 0.63 kg ha^{-1} a.e. plus 3 kg ha^{-1} of ammonium monosulfate in 190 L ha^{-1} of water.

Table 2 Agronomic factors for 2009 and 2010 sites

Agronomic factor	2009	2010
Spring soil sample date	19–21 Apr.	2, 7 Apr.
Spring soil water (mm)[a]	221	183
Spring planting date	13 Apr.	24 Mar.
Spring-planted harvest date		
Flower	1 July	30 June
Intermediate	10 July	–
Pod	17 July	14 July
Summer soil sample date	17 June	21 June
Summer soil water (mm)[a]	223	246
Summer planting date	22 June	21 June
Summer-planted harvest date		
Flower	12 Aug.	11 Aug.
Natural (2009)/Pod (2010)[b]	14 Sep.	27 Aug.
Seeding rate, (seeds m^{-2})		
Pea	80	80
Lentil	120	120
Wheat	250	250
Fertilizer (kg ha^{-1})[c]		
Pea, lentil	6–29–28–10	6–29–28–10
Wheat	11–58–56–19	6–29–28–10

[a] Plant available soil water to 0.9-m depth measured in stubble on or near each planting date

[b] Natural termination

[c] N–P–K–S was applied as a granular 50/50 blend of monoammonium phosphate (11–52–0) and potassium sulfate (0–0–50–17); 2009 spring-planted wheat reference crop strips received double fertilizer due to seeder overlap

Soil was sampled from four locations within each legume plot (near the center of each quadrant) at both seeding and harvest and at two locations within the wheat strip at harvest. Soil cores were extracted to a depth of 0.9 m, divided into 0.3-m depth increments, and subsamples from each location in the plot or strip were mixed by depth. Samples were stored in plastic-coated paper bags and placed in coolers for transport to the laboratory. Aboveground legume and wheat biomass was harvested 3 days after glyphosate application to account for continued crop water use following application. Biomass was collected by clipping plants at the soil surface from two adjacent rows 1 m in length at two locations within each legume plot and wheat strip. Biomass samples were combined in the field for a total collection area of approximately 1 m^2 from each plot and wheat strip. If present, at least two plants of the two dominant non-N-fixing weeds in each plot were collected to increase the number of reference plants. In 2009, *Bromus tectorum*, a weedy annual monocot with both winter and spring growth habits, was collected from 20 of 24 spring-planted plots; weeds were absent from all four pod-terminated pea plots. In 2010, both *B. tectorum* and *Sisymbrium altissimum*, a mustard-family weed, were collected from all spring-planted plots ($n = 16$), and either *Kochia scoparia* or *Amaranthus retroflexus* (both dicot weeds) were collected from 12 of 16 summer-planted plots.

Soils were weighed, dried (50°C, 72 h), and reweighed to determine bulk density and gravimetric water content. Dried soil was ground, passed through a 2-mm sieve and subsamples were extracted for soil NO_3–N with 1 M KCl as outlined by Bundy and Meisinger (1994). Extracts were analyzed using a flow injection analyzer (Lachat Instruments Inc., Loveland, CO, USA). Soil bulk density values were averaged by depth across all plots from the same sampling date and used to calculate soil NO_3–N content (kg N ha^{-1}) from soil NO_3–N concentrations. Aboveground biomass was dried (50°C, 72 h), weighed, and ground (<0.5 mm) and tissue subsamples were analyzed for total N and ^{15}N concentration with a continuous flow isotope-ratio mass spectrometer (Stable Isotope Facility, UC Davis, CA, USA).

Nitrogen fixation

Nitrogen fixation was estimated with the nitrogen difference (ND) and ^{15}N natural abundance (NA) methods (Unkovich et al. 2008) for each legume plot. Wheat was used as the reference plant for both methods and in-plot weeds were used as an additional reference plant for the NA method. Nitrogen fixed by the ND method was calculated as reported by Unkovich et al. (2008):

$$\text{N fixed} = \left(\text{Plant N}_{\text{legume}} - \text{Plant N}_{\text{wheat}}\right) + \left(\text{Soil N}_{\text{legume}} - \text{Soil N}_{\text{wheat}}\right) \quad (1)$$

Soil N in Eq. 1 refers to soil NO_3–N at harvest. It was assumed that soil NO_3–N at seeding was the same for both the legume plot and adjacent reference wheat strip. Mean wheat biomass and shoot N and soil NO_3–N at each harvest for both years are given in Online Resources 1 and 2.

Nitrogen fixation by the NA method was calculated separately using both wheat (NA-wheat) and weeds (NA-weed) as reference plants. The fraction of N derived from the atmosphere via fixation (Ndfa) was calculated as reported by Shearer and Kohl (1986):

$$\text{Ndfa} = \left(\frac{\delta^{15}\text{N}_{\text{reference}} - \delta^{15}\text{N}_{\text{legume}}}{\delta^{15}\text{N}_{\text{reference}} - \text{B}}\right) \quad (2)$$

where $\delta^{15}N$ refers to the atomic abundance of ^{15}N in the sample relative to the atmosphere:

$$\delta^{15}\text{N}(‰) = \left(\frac{\text{atom}\%^{15}\text{N}_{\text{sample}} - \text{atom}\%^{15}\text{N}_{\text{atmosphere}}}{\text{atom}\%^{15}\text{N}_{\text{atmosphere}}}\right) \times 1000 \quad (3)$$

The B in Eq. 2 refers to the $\delta^{15}N$ of lentil (−0.71‰) and pea (−0.73‰) grown in N-free soil conditions, as determined by a sand-culture greenhouse experiment (McCauley 2011). These values are within the range of previously published B values reported for field pea and lentil cultivars (Unkovich et al. 2008). Total aboveground N fixed was calculated by multiplying biomass yield, tissue N concentration as a fraction, and Ndfa. Estimates of N fixation by either the ND or NA method that resulted in Ndfa values less than or greater than the possible range of 0.0–1.0 were set to 0.0 or 1.0, respectively.

Statistical analyses

All statistical analyses were performed using JMP 8.0 statistical software (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed to determine significant treatment differences and interactions at $\alpha \leq 0.05$. Treatment differences were further evaluated using planned orthogonal contrasts and Fisher's protected least significant difference (LSD) tests. Blocks (replicates) were considered a random effect and, when applicable, planting time, termination time, legume species, and year were fixed effects. Site-years were analyzed independently for overall treatment effects on pea and lentil because of differences in planting and termination treatments between years. To determine the effect of year and legume species on dependent variables, site-years were combined for spring-planted crops only (2009 intermediate-termination data excluded) and analyzed

Table 3 Precipitation and temperature data for Amsterdam, Montana, 2009 and 2010 sites

	Precipitation (mm)			Mean temperature (°C)		
	2009	2010	LTA[a]	2009	2010	LTA[a]
Oct.–Apr.[b]	115	110	154	0.8	−1.0	−1.1
May	26	58	57	12.7	9.6	10.5
June	63	86	65	15.4	15.7	15.1
July	56 (25)[d]	18 (13)[d]	29	19.9	19.6	18.9
Aug.	28	57 (22)[e]	29	19.5	18.9	18.3
Sep.	18	30	35	17.2	14.9	12.5
Growing season[c]	192	249	220	16.9	15.7	15.1

[a] Long-term average, 1971–2000 mean annual precipitation for Belgrade, Montana airport located 19 km from the site (Western Regional Climate Center, Desert Research Institute, Reno, NV, USA)

[b] Over-winter preceding the growing season

[c] May-Sept

[d] Monthly precipitation total through spring pod harvest (17 July 2009 and 14 July 2010)

[e] Monthly precipitation total through summer pod harvest (27 Aug. 2010)

using ANOVA. Correlation analyses using Pearson correlation coefficients were performed to compare quantities of N fixed among N fixation methods. Paired t-tests were used to assess differences in reference plant δ^{15}N values collected from the same plot.

Results and discussion

Climatic conditions

Mean precipitation and temperature for the 2 years of this study and the 30-year long-term average are shown in Table 3. In 2009, precipitation during the spring growing season (May to mid-July) was below normal. Notably, May rainfall was 55% less than the month's long-term average. In 2010, spring growing season precipitation was above normal, particularly in June when precipitation was 32% higher than the long-term average. Summer precipitation in 2010 was marked by below average rainfall in July and near normal rainfall in Aug., excluding a large rain event that occurred post-harvest of summer-planted pod treatments in late August. Mean monthly temperatures from May to Aug. 2009 were consistently 1–2°C higher than the long-term average. Mean monthly temperatures throughout the 2010 growing season were similar to the long-term averages.

Legume biomass and N uptake

Biomass yield and shoot N uptake for pea and lentil in 2009 and 2010 are given in Table 4. Biomass yield differed among termination times for both pea and lentil in 2009. Pea biomass increased by 26% between flower and intermediate stages and 65% between flower and pod stages. Lentil biomass production increased by 140% between flower and pod stages. There was no difference in biomass production between flower and intermediate stages for lentil. Shoot N uptake did not differ among termination timings for pea, despite an increase in biomass. Shoot N uptake by lentil was greatest at pod stage and corresponded with increased biomass production. In 2010, there was a significant planting effect, termination effect, and planting by termination interaction on biomass production for both crops. For spring crops, pea and lentil biomass increased 56 and 167%, respectively, from flower to pod stage and shoot N uptake was highest at pod stage for both crops. For summer-planted plots, only lentil biomass increased between flower and pod, though shoot N uptake did not differ between terminations. Across termination times, pea biomass and N uptake were approximately threefold and twofold higher, respectively, for spring-planted than summer-planted pea. This was likely a result of limited precipitation during the summer growing season. Biomass production and shoot N

Table 4 Means and summary of analysis of variance for aboveground dry biomass and shoot N content by legume species and year for each planting and termination treatment

Planting[a]	Termination	Biomass (Mg ha^{-1})		Shoot N (kg ha^{-1})	
		Pea	Lentil	Pea	Lentil
2009					
Spring[a]	Flower	3.0c	1.6b	105a	51b
	Int[b]	3.7b	2.5b	98a	72b
	Pod	4.9a	3.7a	107a	106a
	$\text{LSD}_{0.05}$	*0.7*	*1.1*	*NS*[c]	*30*
Source of variation	*df*	*P* values			
Termination (T)	2	**<0.01**	**<0.01**	0.66	**0.01**
2010					
Spring	Flower	3.2b	1.2bc	116b	45b
	Pod	5.0a	3.2a	147a	98a
Summer	Flower	1.8c	1.1c	47c	30b
	Pod	2.2c	1.8b	44c	39b
	$\text{LSD}_{0.05}$	*0.7*	*0.6*	*22*	*21*
Source of variation	*df*	*P* values			
Planting (P)	1	**<0.01**	**<0.01**	**<0.01**	**<0.01**
T	1	**<0.01**	**<0.01**	0.08	**<0.01**
P × T	1	**0.01**	**<0.01**	**0.03**	**<0.01**

Columns with the same letter are not statistically different and significant effects are shown in bold ($P < 0.05$)

[a] Only spring-planted treatments were analyzed in 2009

[b] Intermediate termination time

[c] Not significant

uptake were greatest for spring-planted lentil terminated at pod; there was no difference between spring-planted lentil terminated at flower and summer-planted lentil terminated at flower or pod.

A strong positive correlation between biomass production and N uptake has been reported for both lentil and pea (Biederbeck et al. 1996; Unkovich et al. 2010); however, the rate of N accumulation by legumes, either by N fixation or soil N uptake, has been shown to decline during early reproductive stages and can be related to water stress (Jensen 1987; Salon et al. 2001). Less precipitation in 2009, particularly in late June and early July, may have contributed to the cessation in pea N uptake after flower. Izard (2007) also reported that spring pea shoot N did not differ between flower and pod in Montana and attributed it to scarce rainfall between the two stages.

Comparison of N fixation methods

Nitrogen fixation for pea and lentil as estimated by the ND, NA-wheat, and NA-weed methods resulted in a range of N fixed values for each treatment combination; however, treatment effects on N fixation were often in agreement among the three methods, particularly between the ND and NA-weed methods (Table 5). A correlation analysis of N fixed by the two NA methods and the ND method revealed a significant positive linear relationship between methods in 2009 and 2010 (Fig. 1). Of the two NA methods, the NA-weed method was more closely correlated with the ND method than the NA-wheat method in both years. In addition, the NA-wheat method was less reliable in that it estimated N fixation amounts less than zero in 5 of 24 plots in 2009 (legume δ^{15}N > wheat δ^{15}N; N fixed estimates set to zero). By comparison, the NA-weed method resulted in only one N fixed estimate less than zero in 2009 and N fixed estimates were always greater than zero for the ND method. All N fixation estimates were greater than zero in 2010.

Two factors that apparently affected the accuracy and precision of the NA-wheat method, particularly in 2009, were the small observed differences between atmospheric ^{15}N and soil ^{15}N uptake (Bremer et al. 1993), and the spatial and temporal variability of the ^{15}N abundance of soil available N (Hauggaard-Nielsen et al. 2010; Walley et al. 2001). A δ^{15}N enrichment difference of at least 2‰ between the reference plant and N-fixing plant has been recommended for acceptable precision of N fixation estimates by the NA method (Unkovich et al. 1994). In both years of this study, mean differences in δ^{15}N values between wheat and legumes at each harvest were less than 2‰ and

Table 5 Means and summary of analysis of variance for nitrogen (N) fixed as determined by the N difference (ND) and ^{15}N natural abundance (NA) methods by year and legume species for each planting and termination treatment

Planting[a]	Termination	N fixed (kg N ha^{-1})					
		ND		NA-wheat		NA-weed[a]	
		Pea	Lentil	Pea	Lentil	Pea	Lentil
				2009			
Spring[b]	Flower	84.0a	35.2b	47.4a	21.7b	53.9a	16.4b
	Int[c]	74.3a	49.6ab	18.8a	13.4b	62.5a	29.2b
	Pod	75.2a	74.3a	21.0a	46.2a	na[d]	61.3a
	LSD$_{0.05}$	*NS*[e]	*24.8*	*NS*	*14.7*	*NS*	*32.0*
Source of variation	*df*			*P* values			
Termination (T)	2	0.63	**0.02**	0.13	**<0.01**	0.57	**0.03**
				2010			
Spring	Flower	106.0b	24.6b	78.6a	20.8ab	95.0b	31.0b
	Pod	135.7a	89.1a	85.6a	35.0a	123.8a	72.9a
Summer	Flower	32.2c	12.9b	26.5b	17.1b	43.5c	17.9b
	Pod	26.5c	20.3b	30.2b	30.0ab	35.4c	32.0b
	LSD$_{0.05}$	*28.7*	*16.7*	*17.0*	*16.5*	*21.8*	*23.5*
Source of variation	*df*			*P* values			
Planting (P)	1	**<0.01**	**<0.01**	**<0.01**	0.41	**<0.01**	**<0.01**
T	1	0.21	**<0.01**	0.34	**0.03**	0.20	**<0.01**
P x T	1	0.08	**<0.01**	0.76	0.90	**0.04**	0.06

Columns with the same letter are not statistically different and significant effects are shown in bold ($P < 0.05$)

[a] Predominant weed reference crop for each year and planting were as follows: 2009/spring, *Bromus tectorum*; 2010/spring: *Sisymbrium altimissum*; 2010/summer: *Kochia scoparia* and *Amaranthus retroflexus*

[b] Only spring-planted treatments were analyzed in 2009

[c] Intermediate termination time

[d] Not available due to absence of weeds in these plots

[e] Not significant

wheat δ^{15}N values were consistently less than weed δ^{15}N values (Table 6). Subsequently, the NA-weed method resulted in higher observed N fixed values and greater method precision than the NA-wheat method, particularly in 2010 when mean δ^{15}N differences between broadleaf weeds and both legumes consistently exceeded 2‰. Differences in δ^{15}N values among reference species grown in close proximity have been observed by a number of investigators (Bremer et al. 1993; Hauggaard-Nielsen et al. 2010; Houngnandan et al. 2008; Pate et al. 1994) and attributed to different isotopic discrimination among species, dissimilar rates of plant N uptake throughout the growing season, and different rooting patterns. These are all possible reasons for the observed differences in N fixation by the NA-wheat and NA-weed methods in this study and further emphasize the importance of using multiple reference plants when utilizing the NA method (Unkovich et al. 2008). In addition, there was better agreement among methods in 2010 than 2009, indicating site and/or soil conditions were a factor affecting N fixation estimates among methods.

Regardless of potential inaccuracies by the ND and NA methods to quantify N fixation, the primary objective of this work was to evaluate treatment effects on N fixation. Treatment effects on N fixed as determined by the ND and NA-weed methods were in close agreement for both years of this study, strongly suggesting that the measured differences were valid. Based on the high ^{15}N variability observed, incomplete weed data in 2009, and low expected soil N losses in a semi-arid climate, estimates of N fixation by the ND method were believed to be more reliable

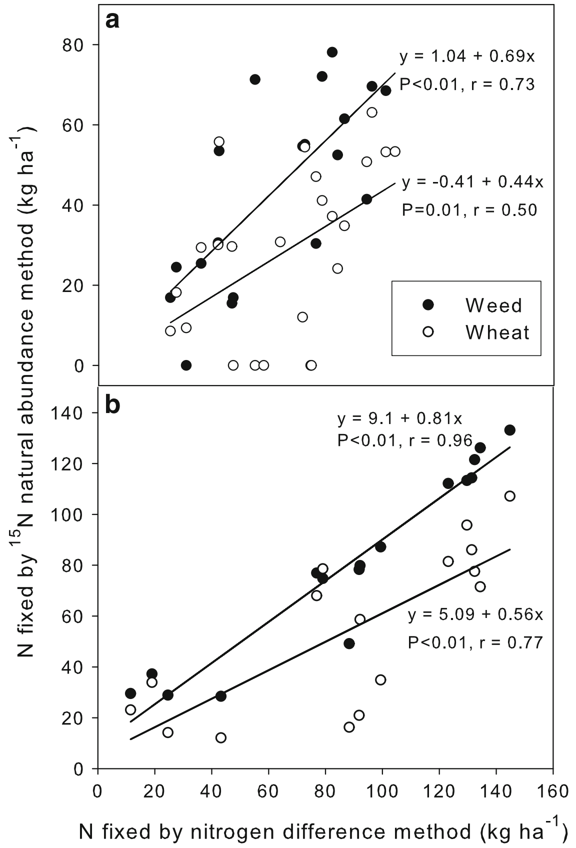

Fig. 1 Correlation between nitrogen (N) fixed by the N difference (ND) method and the ^{15}N natural abundance (NA) method for spring-planted legume crops in 2009 (**a**) and 2010 (**b**) using weeds (*filled circles*) or wheat (*open circles*) as the reference plant for the NA method. Each point represents data from an individual plot

than estimates by the NA method in this study. Therefore, treatment effects and specific N fixation amounts are only discussed for the ND method.

Treatment effects on N fixation

In 2009, termination timing had a significant effect on N fixed by lentil, but not pea. Nitrogen fixed by lentil more than doubled between flower and pod. In contrast, N fixed by pea was the same among the three terminations in 2009. In 2010, there was a significant termination effect on N fixation for both spring-planted crops. Pea and lentil fixed 30 and 65 kg ha^{-1} more N by pod than flower, respectively. For summer-planted crops, there were no differences in N fixed between flower and pod. Planting time affected the amount of N fixed in 2010 with spring-planted crops fixing considerably more N than summer-planted crops in three of the four treatments. The exception was lentil terminated at flower stage which fixed similar amounts of N in spring and summer, resulting in a significant planting time by termination time interaction. Mean soil NO_3–N levels in the top 0.9 m were lower at summer-planting than spring-planting; therefore, nodulation in summer-planted crops was likely not delayed by high levels of available soil N. Rather, low precipitation and higher evaporation during the summer growing season likely decreased biomass production and N fixation of the summer-planted crops.

When 2009 and 2010 spring-planting N fixation data sets were combined, year, legume species, and termination time had a significant effect on N fixation and all two-way interactions were significant ($P < 0.001$; combined data not shown). For pea, cooler and wetter growing conditions in spring 2010 led to greater biomass production and crop N demand, which in turn increased the amount of N fixed at pod by approximately 80% compared to 2009. Lentil N fixation amounts were similar between years at both

Table 6 Mean tissue δ^{15}N (‰) for legumes, wheat, and collected weeds in 2009 and 2010

Planting	Termination	Pea	Lentil	Wheat	Weed[a]
			2009		
Spring	Flower	0.49	0.51	1.51	1.58
	Int[b]	0.57	0.78	0.98	2.27
	Pod	0.80	0.32	1.13	1.89
			2010		
Spring	Flower	−0.13	0.19	1.34	2.90
	Pod	−0.09	0.36	1.11	3.60
Summer	Flower	−0.34	−0.04	0.55	3.31
	Pod	−0.36	−0.31	0.71	2.18

[a] Weed species were *Bromus tectorum* (2009), *Sisymbrium altissimum* (2010 spring), and *Kochia scoparium* or *Amaranthus retroflexus* (2010 summer)

[b] Intermediate termination time

flower and pod stages. Between legume species, the greatest difference in N fixed occurred at flower stage with pea fixing 2.4 and 4.3-fold more N than lentil in 2009 and 2010, respectively.

There is a well-established correlation between biomass production and N fixation for a number of leguminous crops (Unkovich et al. 2010). This relationship was observed for lentil in both years of this study and 1 year for pea. In 2009, spring-planted pea terminated at intermediate and pod stages appeared to have experienced enough water-stress to cease N fixation, yet not biomass production. Nitrogen fixation has been reported to be more sensitive to water stress than biomass production and N assimilation for a number of legume crops, and can be affected by both the severity and timing of water stress (Castellanos et al. 1996; Kirda et al. 1989; Serraj et al. 1999). A two-year, multisite dryland study in Saskatchewan found N fixation by pea in a drought year to be reduced by 40% on average across two sites compared to N fixed at the same sites in the following year with more normal precipitation (Bremer et al. 1988). Kirda et al. (1989) reported a decrease in N fixation in soybean by almost 60% on average following a mild water stress period compared to non-water stressed plants, though biomass production was not different between treatments. The timing of water stress relative to crop development can also influence N fixation (Thomas et al. 2004). This may have partially caused N fixation by pea to be more affected by water stress than lentil N fixation in spring 2009 because pea had depleted soil water more than lentil by flower (data not shown).

Conclusions

Choosing when to plant and terminate a LGM crop can affect biomass production and the amount of N fixed by the crop. In this 2-year field study, spring-planting resulted in substantially higher biomass yield and N fixed for pea and lentil compared to summer-planting as a result of reduced precipitation during the summer growing season. Fall-planted legume crops failed to establish sufficiently in this study and further research will be needed to assess the effect of fall-planting on N fixation. Pod termination resulted in peak biomass production and N fixation for both legume crops when spring precipitation was above normal. Yet, when growing season precipitation was below normal and inconsistent, N fixed by pea did not increase beyond flower stage despite an increase in biomass. Findings from this research indicate that delaying termination beyond flower can result in additional N fixation, but only when sufficient water is available. In growing seasons with below normal or inconsistent precipitation patterns, early termination may result in similar N fixation amounts as a later termination. Not only would there be no additional N fixation gain from a later termination, but also a greater effect on soil water content, particularly in a drier year. Results from this study should prove useful in balancing N gains and soil water losses when managing LGMs in dryland cropping systems.

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