



Cover crop effects on the fate of N in sweet maize (*Zea mays* L. *saccharata* Sturt.) production in a semiarid region

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Abstract

This research aimed to determine the effects of different cover crops and application of bio-fertilizer on dynamic of nitrogen in the soil and sweet maize yield. Also, we evaluated the effect of fall–winter species (common vetch, field pea, winter oats, fodder kale) and a mixture of vetch and field pea with oats used as cover crops, as such as dead organic mulch and traditional variant, without coverage on biomass, chlorophyll and protein content in leaves and grain of main crop. Biomass production and N uptake by cover crops ranged from 4.25 to 90.20 kg ha⁻¹ and from 0.34 to 133.80 kg ha⁻¹ N, respectively, depending on cover crop type. At harvest soil nitrate content in treatments with cover crops was 50-90% lower than in the control, reducing spring N leaching risk. Residual mineral N significantly increased with application of microbiological fertilizer. The chlorophyll content of the main crop was significantly lower in treatments without cover crops. Consequently, sweet maize yield was the highest in fodder kale and field pea (7263.83 and 7177.27 kg ha⁻¹) treatments, but the smallest in winter oat and common vetch (6802.47 and 6184.14 kg ha⁻¹). In terms of all investigated traits, particularly grain yield, cover crops and microbiological fertilizer expressed more efficiency in the dry year. It could be concluded that N content should be controlled effectively by sowing main crops after planting of cover crops in biological farming systems in a semiarid region.

Keywords: Sweet maize; Cover crops; N fate; Microbiological fertilizer; Yield.

Introduction

The lack of information on sweet maize cultivation practices limits its introduction into temperate climate zones. In production of sweet maize, environmental pollution by N residues after crop harvesting is possible (Silgram and Shepherd, 1999). It includes the residual soil mineral N (N_{min}) and N in crop residues (Neeteson et al., 1999).

Cover crops have a very important role in improving the health of soil (Wang et al., 2011), prevention of erosion (Mazzoncini et al., 2011), protection of water quality (Malone et al., 2014) and biological diversity (Castro-Caro et al., 2014). If there is not enough nitrogen in nested organic mass, the microorganisms will use the mineral nitrogen from the soil (Mahdi et al., 2010). Particularly, pronounced differences in yield of the main crop were detected when it was grown on cover crops compared to the bare soil (without vegetation). Dolijanovic et al. (2012) state, that the lowest grain yield of

sweet maize and the smallest shelling percentage was achieved in the conventional system, while higher yields were noted from plots with legume species, winter hairy vetch and non-legume species, like winter fodder kale used as after crop. Similar results were recorded by Uchino et al. (2009), who cultivated soybeans as a main crop in crop rotation with maize, after a winter cover crop.

Managing manure in cropping systems to retain nutrients and prevent adverse off-site impacts is difficult challenge, especially related to managing N losses (Parkin et al., 2006). The cover crops can reduce the losses of nitrogen from agricultural systems by reducing the nitrate leaching and evaporation of the ammonia and the nitrogen oxides to the atmosphere. Restovich et al. (2012) studied the effect of different cover crops from the *Poaceae*, *Fabaceae* and *Brassicaceae* families and grass-legume mixtures on the content of NO_3^- -N in the soil. After removing of cover crops the NO_3^- -N content in the soil was 50-90% lower than in the control plots. Reduction of NO_3^- -N in soil illustrates the ability of cover crops to reduce nitrogen loss through leaching during periods when precipitation exceeds evapotranspiration values or until the soil is uncovered by crop (Constantin et al., 2010). In semiarid regions in Serbia, Oljaca and Dolijanovic (2013) found a significant variation in the content of NO_3^- N depending on the type of cover crop, especially in a layer of 0.20 - 0.40 m.

Bio-fertilizers have an important role in keeping high soil fertility and crop yields increasing (Mahdi et al., 2010). The positive impact of microbiological fertilizers is also observed in regards to quality of plant products, as for example increase in lycopene and vitamin C accumulation in tomato fruits (Verma et al., 2015; Ochoa-Velasco et al., 2016) and higher glutathione content in maize grain (Dragicevic et al., 2013).

Data obtained in this study from field experiments provides valuable knowledge regarding the (i) influences of different type of cover crops and (ii) the applied form of fertilizers (i.e. microbiological) on fate of N in soil and on morphological, grain yield and chemical composition of stem and grain of sweet maize.

Material and Methods

A field experiment was carried out in 2013/14–2014/15 growing seasons, at the Experimental Field of Maize Research Institute in Zemun Polje near Belgrade (44° 52' N; 20° 20' E). The soil was slightly calcareous chernozem with 47% of clay and silt and 53% of sand. The soil at 0-30-cm layer were contained 3.22% of organic matter, 0.19% of total N, 1.9% of organic C, 16.2 and 22.4 mg per 100 g soil of available P and extractable K, respectively, 1.38% of total CaCO_3 and had pH 7.3.

The experiment was established as a block design with four replications. As winter cover crops (factor A) the following plants were grown: CV–common vetch (*Vicia sativa* L.), FP–field pea (*Pisum sativum* L.), WO–winter oats, (*Avena sativa* L.), FK–fodder kale (*Brassica oleracea* (L.) *convar. acephala*), two mixture variants of legume crops with oats (CV+WO and FP+WO) and two control treatments: a variant in which the surface was covered with dead organic mulch (DOM) and traditional variant: after ploughing in the fall plot stayed uncovered during the winter (TV). The cover crops (CC) were sown in the amount: common vetch – 120 kg, field pea – 150 kg, oat – 160 kg and fodder kale 15 kg per ha and in mixture relation between legume and oats was 70:30. The plot size was 17.5 m². The seeds of the Institute for Forage Crops Institute of Field and Vegetable Crops in Novi Sad was used for planting in both years. The seeds of sweet maize 'ZPSC 421su (FAO 400) were sown at the arrangement of

70 cm between rows and 22 cm between plants in the row (65,000 plants per ha). Preceding crop in both years was winter wheat. The autumn soil preparation (ploughing and seedbed preparation) was performed immediately before sowing, when also soil samples were taken for available N analysis at depths of 0-20 cm and 20-40 cm. Further soil sampling from all CC and control treatments was done in the spring, after CC harvest, as well as after sweet maize harvesting.

Before the sowing of CC (autumn) and sweet maize (spring) mineral fertilization was applied in order to obtain 120 kg ha⁻¹ N, 90 kg ha⁻¹ P and 60 kg ha⁻¹ K. The total amount of P and K fertilizer was applied in autumn with mono-potassium phosphate fertilizer (a.m. 0:52:34) and the required N amount was incorporated together with sweet maize sowing (urea 46% a.m). Nitrogen fertilization followed: for non-legume crops and control treatments it was 120, for sole legume it was 80 and for mixture it was 90 kg ha⁻¹ N. The remaining 40 or 30 kg ha⁻¹ N was considered to be provided by nitrogen fixation.

Green biomass of the cover crops was incorporated in the soil, immediately after, half of the elementary plot was infested with bio-fertilizer (BF) - Uniker (mobilizer of nutrients) in an amount of 10 l ha⁻¹ (factor B), which contains the strains of cellulolytic and proteolytic bacteria to support the mineralization of entered crop residues.

Available N forms (NH₄⁺-N and NO₃⁻-N) were determined by the method Scharpf and Wehrmann (1975). From the morphological parameters, fresh weight of whole plants (biomass) in the anthesis stage (sampled after 2 uniform plants per replication) was measured. Then the chlorophyll content using SPAD meter (Infraneo, Chopin Technologies, France) was measured from 3 plants per each replication, with 3 places on the ear leaf blade, while the kernel protein content was measured on infrared analyser.

The ears were harvested at the stage of milk maturity of kernels. The schedule of the main works on the experiment is shown in Table 1. The obtained data were processed using analysis of variance for two-factorial experiments (ANOVA). For the individual comparisons, the least significant difference (LSD test) was used. Principal component analysis (PCA) was used for evaluation of interdependence between factors analyzed, including subtraction in available N content between sowing and harvesting time. Statistical analysis was performed by SPSS 15.0 (IBM Corporation, Armonk, New York, USA) for Windows Evaluation version.

Table 1. Chronology of field operations and length of vegetation period of sweet maize.

Cover crops sowing	October, 30 2013	November, 13 2014
	2014	2015
Cover crops sampling	April, 23	May, 12
Cover crops and microbiological fertilizer incorporated	May, 12	May, 21
Sweet maize sowing	May, 20	May, 21
Hand weeding 1	June, 27	June, 22
Hand weeding 2	July, 17	July, 15
Sweet maize harvest	August, 14	August, 21
Length of vegetation period of sweet maize (in days)	86	92

Results and Discussion

The difference in sweet maize biomass influenced by the CC and the CC*BF interaction was statistically significant in both years (Table 2). In the first year, cover crops compared to TV and DOM expressed an effect on the maize biomass only in treatments with bio-fertilizer. The importance of cover crops for sweet maize growth was particularly manifested in the second year of investigation. The highest yields of biomass were found in variant with fodder kale and field pea (53.8 and 53.0 kg ha⁻¹) and the lowest in DOM and TV (44.0 and 42.5 kg ha⁻¹-Table 3). The chlorophyll content in the sweet maize leaves ranged from 36.25 (2015 without BF) to 52.64 (2014 with BF), with statistically significant differences between CC treatments in both years. Compared to TV all variants of cover crops, except for common vetch, resulted in the higher chlorophyll content in maize leaves. The application of bio-fertilizer increased chlorophyll content in both years, but a statistically significant difference was achieved only in 2014 (Table 2).

Table 2. The biomass of sweet maize plants and the chlorophyll amount in maize leaves.

Treatment	Biomass (kg/ha)					Chlorophyll (SPAD units)				
	2014		2015		Average	2014		2015		Average
	BFØ	BF	BFØ	BF		BFØ	BF	BFØ	BF	
CV	88	94	4	3	47.2	50.03	55.47	26.60	28.19	41.95
FP	99	105	3	5	53.0	49.32	53.09	37.37	35.21	43.75
WO	91	75	6	3	43.8	48.40	51.56	38.29	37.76	44.00
FK	102	101	6	6	53.8	52.89	54.84	38.06	38.54	46.08
CV+WO	88	101	3	5	49.2	50.44	53.36	39.53	38.57	45.48
FP+WO	85	81	5	6	44.2	46.40	50.35	35.89	39.78	43.10
DOM	84	82	5	5	44.0	47.29	50.46	42.31	40.71	45.19
TV	82	83	2	3	42.5	47.49	52.01	31.97	38.20	42.42
Average	89.9	90.2	4.25	4.50	47.3	49.03	52.64	36.25	37.12	44.00
LSD 0.05 Biomass 2014	CC**		BF ^{ns}	CC × BF*	LSD 0.05 Chlorophyll 2014	CC**		BF**	CC × BF ^{ns}	
	0.86		0.43	1.21		1.27		0.63	1.80	
LSD 0.05 Biomass 2015	CC**		BF ^{ns}	CC × BF*	LSD 0.05 Chlorophyll 2015	CC**		BF ^{ns}	CC × BF**	
	0.78		0.39	1.11		2.46		1.23	3.48	

P<0.01 very significant (**); P<0.05 significant (*); P>0.05 no significant (^{ns}).

The investigated factors (CC and BF) showed significant effect on protein content in sweet maize kernel in both years (table 3). As it was expected, the greatest impact on protein content was exhibited in leguminous species grown alone, or in mixtures with oats, particularly in the dry, 2015. Small grains intercropped with legumes obtained higher values of protein content than small grain grown as monocrops (Kadžiuilienė et al., 2011).

Table 3. The protein content (%) in sweet maize grain.

Treatments	2014			2015		
	BFØ	BF	Average	BFØ	BF	Average
CV	10.92	10.87	10.58	11.68	11.16	11.42
FP	11.23	10.68	10.96	11.54	11.36	11.45
WO	10.68	10.09	10.39	11.56	10.04	10.80
FK	11.08	10.79	10.94	11.18	10.87	11.03
CV+WO	11.14	11.82	11.48	11.32	11.33	11.33
FP+WO	10.22	10.14	10.18	10.58	11.64	11.11
DOM	10.80	10.29	10.55	10.42	10.27	10.35
TV	11.39	10.81	11.10	11.01	11.36	11.19
Average	10.93	10.68	10.81	11.16	11.00	11.08
LSD 0.05	CC**	BF**	CC × BF**			
Protein content 2014	0.063	0.032	0.090			
LSD 0.05	CC**	BF**	CC × BF**			
Protein content 2015	0.021	0.010	0.029			

P<0.01 very significant (**); P<0.05 significant (*); P>0.05 no significant (^{ns}).

The nitrate content in the soil depends on the fertilizers application, crop residues, mineralization, i.e. microbial activity and their leaching into deeper soil layers. Nitrate N in the soil, regardless of origin, is highly soluble and mobile; it could be bound by biological systems and by ascendant and descendent pathways can be washed to deeper layers. Ammonium N form is mostly fixed to the soil colloids and its losses are through the volatilization or of denitrification. Ammonia N content increase is accompanied by an increased content of nitrate N (Kastori, 2005).

Data in Tables 4 and 5 clearly shows that the content of available N forms differs in the first and second year of investigation. This research has shown, that cultivation of cover crops can reduce the leaching of nitrates to the deeper soil layers. The highest amount of NH_4^+ -N was accrued with FP+WO crop (1.63 kg ha^{-1}) in spring and in treatment with field pea (2.24 kg ha^{-1}) after sweet maize harvest in depth of 20 cm. NO_3^- -N accumulated greater in depth of 20-40 cm than in the upper layer, especially in the second year of investigation. Cover crops can be used to reduce NO_3^- leaching by immobilizing soil inorganic N into plant biomass during periods of excess water (Constantin et al., 2010) and to reduce crop fertilization by supplying the utilized N to the succeeding harvest crop through mineralization of residues (Uchino et al., 2009), but if N from cover crops is not released synchrony with the harvest crop demand, it may be lost through leaching (Sainju et al., 2007). In the case of vetch, this may have been related to the dry conditions during the 2015 growing season and particularly during flowering, when N uptake and yielding were reduced. Some studies suggest, that un-irrigated (rain-fed) crops recover more N from fertilizers than from leguminous residue mineralization, increasing residual N at harvest. Cover crop and cover crop biomass management affects the N content of the whole aboveground biomass and maize grain yield and the differences between actual and critical N concentrations in the whole aboveground maize yield (Kramberger et al., 2014).

Table 4. The N content (NH_4^+ and NO_3^-) in the soil before sowing of cover crops, before sowing and after harvest of sweet maize (2013/14).

Time of measure/treatments		CV	FP	WO	FK	CV+WO	FP+WO	DOM	TV	
Content of NH ₄ ⁺ - N (kg/ha)										
Autumn, 2013	0-20					1.82				
	20-40					0.70				
Spring, 2014	0-20	0.63	0.67	0.53	1.29	0.51	1.63	0.74	1.30	
	20-40	0.22	0.66	0.39	0.77	0.33	0.82	0.28	1.10	
After harvest	BFØ	0-20	1.45	1.79	1.93	2.43	1.62	2.24	2.00	1.92
		20-40	0.34	0.72	0.12	0.72	0.19	0.38	0.21	0.71
	BF	0-20	1.60	2.24	2.24	1.70	2.21	1.45	1.64	1.65
		20-40	0.26	1.32	0.22	0.12	0.08	0.38	0.01	0.05
Content of NO ₃ ⁻ - N (kg/ha)										
Autumn, 2013	0-20					42.55				
	20-40					20.96				
Spring, 2014	0-20	19.91	22.59	18.59	25.45	15.76	30.00	14.88	6.19	
	20-40	6.53	14.90	4.40	1.32	9.03	11.62	0.07	1.34	
After harvest	BFØ	0-20	35.85	57.50	46.19	40.21	77.66	52.42	69.08	33.45
		20-40	8.47	41.38	0.34	8.95	7.93	6.40	17.51	13.82
	BF	0-20	34.57	75.43	52.09	70.78	66.26	41.73	74.53	37.36
		20-40	17.57	26.28	19.90	9.97	17.09	15.28	34.63	7.72

Table 5. The N content (NH_4^+ and NO_3^-) in the soil before sowing of cover crops, before sowing and after harvest of sweet maize (2014/15).

Time of measure/treatments		CV	FP	WO	FK	CV+WO	FP+WO	DOM	TV	
Content of NH ₄ ⁺ - N (kg/ha)										
Autumn, 2014	0-20					0.75				
	20-40					0.60				
Spring, 2015	0-20	0.00	0.39	0.90	1.68	1.17	2.29	1.14	0.30	
	20-40	1.19	1.21	0.97	1.15	1.16	1.45	0.47	0.98	
After harvest	BFØ	0-20	1.04	0.45	0.99	0.32	0.92	0.98	0.80	0.45
		20-40	1.78	0.38	0.31	0.13	0.88	0.83	0.73	0.34
	BF	0-20	0.54	0.64	0.90	0.89	0.62	0.60	0.47	0.26
		20-40	0.58	0.58	0.67	0.70	0.79	0.28	0.42	0.12
Content of NO ₃ ⁻ - N (kg/ha)										
Autumn, 2014	0-20					9.10				
	20-40					35.00				
Spring, 2015	0-20	20.68	20.49	14.48	46.38	64.29	36.64	30.75	5.05	
	20-40	85.94	59.47	40.02	26.90	22.98	78.97	61.69	33.61	
After harvest	BFØ	0-20	65.26	28.66	39.15	53.81	33.23	44.92	53.53	16.96
		20-40	36.34	115.34	81.07	133.8	119.57	120.07	38.65	33.26
	BF	0-20	37.02	34.74	102.03	81.25	73.46	60.82	45.53	28.69
		20-40	73.77	117.65	91.97	122.0	120.36	62.59	43.21	49.47

The kernel yield was significantly higher in 2014 than in 2015 (Table 6). Bio-fertilizer and leguminous CC were the best stimulants of crop performance in terms of yield. The CC effect in comparison to the control variants was more pronounced in the second year of investigation, especially in case of robust type of plants (FP, FK and mixtures), interacting with the application of microbial fertilizers and producing significantly higher yields. Application of bio-fertilizer showed the statistical significance on kernel yield in both years. Numerous investigations revealed a positive effect of ploughed spring and winter CC on sweet maize yielding (Uchino et al., 2009; Dolijanovic et al., 2012; Rosa, 2014). It was found that effect of catch crops on yielding potential depends on the species of crop cultivated for ploughing, the amount of biomass and the ploughing date (Sainju et al., 2007).

Table 6. Yield of sweet maize kernels (kg/ha).

Year/Treatments	2014			2015			Average
	BFØ	BF	Aver.	BFØ	BF	Aver.	
CV	7940.37	8655.38	8297.88	4410.17	3730.61	4070.39	6184.14
FP	8434.63	9049.52	8742.08	5076.85	6148.06	5612.46	7177.27
WO	7330.31	8701.45	8015.88	4648.85	6529.26	5589.06	6802.47
FK	8172.42	8359.48	8265.95	6602.47	5920.93	6261.70	7263.83
CV+WO	8377.90	9051.15	8714.53	4290.71	4786.33	4538.52	6626.53
FP+WO	8309.52	8475.97	8392.75	5792.68	5074.11	5433.40	6913.08
DOM	8476.52	8939.19	8707.86	4180.51	6554.72	5367.62	7037.74
TV	8719.30	8934.48	8826.89	5605.98	4232.43	4919.21	6873.05
Average	8220.12	8770.83	8495.48	5076.03	5372.06	5224.04	6859.76
LSD 0.05	CC ^{ns}	BF ^{**}	CC × BF ^{ns}				
Yield 2014	799.73	399.86	1130.98				
LSD 0.05	CC ^{**}	BF [*]	CC × BF ^{**}				
Yield 2015	848.64	424.32	1200.16				

P<0.01 very significant (**); P<0.05 significant (*); P>0.05 no significant (ns).

According to PCA, first two axes explained 40.94% and 29.48%, respectively of total variability for the variables set observed (for biomass, chlorophyll content, protein content in kernel, kernel yield and difference in available N content in soil between sowing and harvesting, respectively). Projection of the variables indicated, that for difference in available N content in soil and chlorophyll content contributed reversely, mainly to PC1 (-0.750 and 0.84, respectively; Figure 1), whereas PC2 was defined with fresh weight (0.91) and protein content in kernel (0.79). This could mean that chlorophyll content in maize leaves is dependable on N content in soil, i.e. its utilization, accordingly to the results of Boggs et al. (2003) who find significant correlation between chlorophyll content in cotton leaves and soil nitrate N. As Uribe-larrea et al. (2007) showed vegetative biomass and grain N concentration in maize depends significantly on soil N rate and genotype, supporting connection between shoot biomass and protein content in kernel. The difference in soil N content between sowing and harvesting was mainly affected by common vetch + Uniker combination, fresh

weight was affected by common vetch + winter oats and field pea + Uniker treatments, kernel yield was influenced by field pea + winter oats + Uniker combination, while kernel protein content was affected mainly by field pea treatment. This could underline importance of bio-fertilizer, as well as field peas as CC for biomass and yield forming, based on increased N availability and its acquiring.

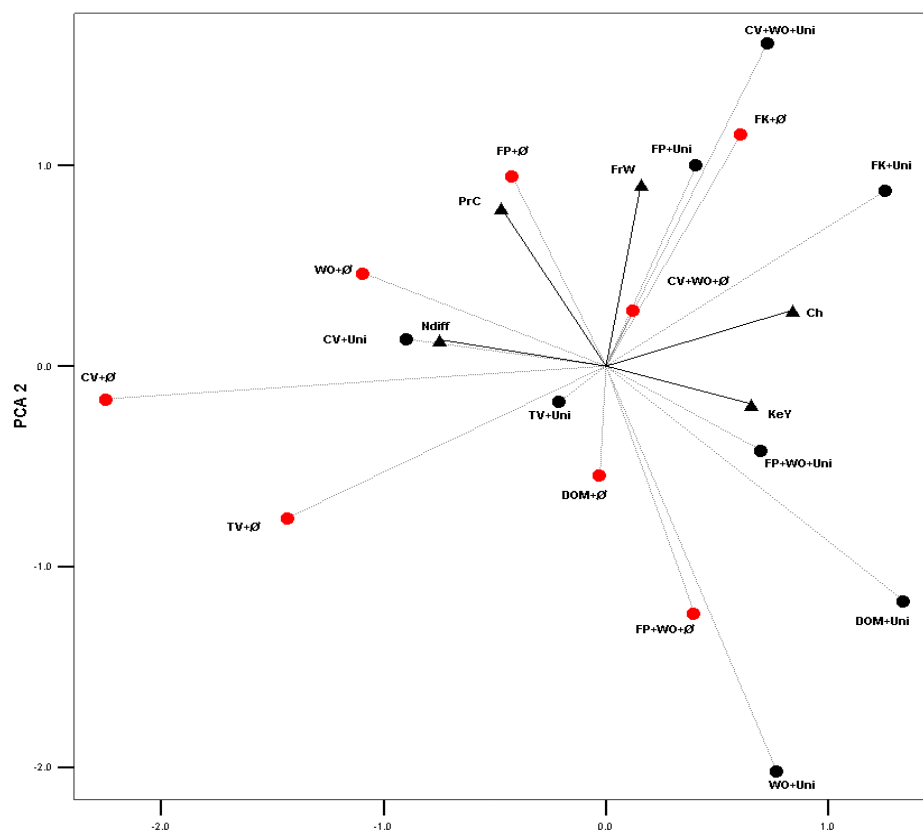


Figure 1. Principal Component Analysis for biomass (FrW), chlorophyll content (Ch), protein content in kernel (PrC), kernel yield (KeY) and difference in available N content in soil between sowing and harvesting (Ndiff) in treatments: CV–common vetch, FP–field pea, WO–winter oats, FK–fodder kale, mixtures: CV+WO and FP+WO, variant with dead organic mulch (DOM) and uncovered variant (TV), in combination with Uniker (BF) or without it (BFØ).

Tested catch crops affected significantly biomass and chlorophyll content of sweet maize plants, emphasizing leguminous crops, sole or in combination with oat. Uniker slightly decreased protein content, but cover crop treatments expressed various effects, with the highest impact of common vetch and field pea on protein content increase. Irrespective that higher yields were obtained in the first year of investigation, cover crops expressed significant and positive effect on yielding in dry, 2015 year, indicating importance of cover crops from the sense of drought frequency and severity in the future. The kernel yield and the apparent remaining N in the soil after maize harvesting, showed significant interaction responses to cover crop $\times$ management, indicating positive and negative effects. Winter cover crops minimize N leaching and legume-cereal mixtures are more effective in soil N management (Robačar et al., 2016). Considering the expected ecological advantages of the cover crops as monocrops or mixtures, the results thereby support their use.

From this study we conclude that the inclusion of some species as cover crops in the simplified cropping systems which actually predominate in the semiarid regions, improves N use efficiency, compared to the alternative long fallow periods between summer crops. Despite the clear benefit of cover crops, additional work must resolve the apparent interactions of fertilizer and of cover crops on N mineralization/immobilization processes.

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