



ORIGINAL PAPER

**EFFECT OF A SOWING REGIME AND WATER
CONDITIONS ON NITROGEN CONTENT
AND ACCUMULATION IN THE AERIAL BIOMASS
OF SPRING BARLEY (*HORDEUM VULGARE* L.)
AND ITALIAN RYEGRASS
(*LOLIUM MULTIFLORUM* LAM.)***

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ABSTRACT

Competitive interactions are common in plant communities, but the underlying mechanisms and effects of competition have not been fully elucidated to date. A pot experiment (3 series in 2009-2011) was conducted to evaluate the effect of a sowing method of spring barley and Italian ryegrass, and different water condition on the nitrogen (N) content and accumulation in the aerial biomass of plants at different stages. The experimental factors were as follows: 1) sowing regime – pure sowing and mixed sowing – spring barley undersown with Italian ryegrass, 2) water supply – plants supplied with water to meet their full requirements and 50% water supply reduction. The N content of the aerial biomass of plants was determined at five phenological development stages of spring barley grown in a pure stand under optimal soil moisture conditions: leaf development, tillering, stem elongation, heading and ripening. Nitrogen uptake was determined based on the N content and dry matter accumulation in plants. Nitrogen concentrations in the aerial biomass of barley and ryegrass varied throughout the growing season. Nitrogen content was particularly high in leaves, and it decreased steadily during plant development. Neither the sowing regime nor the water supply exerted significant effects on the N content of the aerial biomass of spring barley. In Italian ryegrass, under optimal water supply, mixed sowing with spring barley decreased N concentrations in shoots only during tillering. In spring barley, mixed sowing reduced N uptake by biomass, and the observed decrease was exacerbated under water deficit. In Italian ryegrass, reduced water supply decreased N uptake, but the effect of competitive interactions between the analyzed species was usually stronger than the influence of water deficit. Differences in N accumulation in aerial biomass were determined by the

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amount of biomass produced in mixed stands and the responses of barley and ryegrass to water deficit.

Keywords: undersown crop, competition, water deficit, nitrogen content, nitrogen uptake.

INTRODUCTION

Spring barley is considered to be a good companion crop for undersown crops such as legumes, grasses and legume-grass mixtures. After harvest of the companion crop, undersown species exert protective effects and prevent nitrogen leaching from soil (THOMSEN 2005, KÄNKÄNEN, ERIKSSON 2007, MALCOLM et al. 2015). Their aerial biomass can be used as animal feed and post-harvest residues are a valuable source of organic matter rich in nutrients (PŁAZA et al. 2009). Italian ryegrass, which is grown for green fodder, can also be used as undersown crop (GRYGIERZEC, RADKOWSKI 2006).

There is high biodiversity in agroecosystems (JASKULSKI, JASKULSKA 2012). The mechanisms underlying the interactions between plants, observed during the growing season, remain insufficiently investigated. Intraspecific and interspecific competition are forms of competition in which individuals of the same or different species, respectively, compete for limited environmental resources. Italian ryegrass and other species of the genus *Lolium* can be aggressive competitors for cereals. The presence of ryegrass can lead to a considerable decrease in barley grain yield (GONZALEZ PONCE, SANTÍN MONTANYÁ 2007, KÄNKÄNEN, ERIKSSON 2007, GALON et al. 2011). An absence of significant interactions is rarely encountered in such plant communities (LEMOLA et al. 2000, THOMSEN 2005).

Water is an important limiting factor in agricultural ecosystems. The plant's response to water stress is a species-specific or even a variety-specific trait (NORRIS, THOMAS 1982, OLSZEWSKA et al. 2010, PECIO, WACH 2015). Due to the complex effects exerted by various environmental factors, water deficit affects, among others, nutrient uptake (AKMAL et al. 2010, GONZALEZ-DUGO et al. 2010) and changes in nutrient concentrations in plants (GONZALEZ PONCE, SANTÍN MONTANYÁ 2007, GONZALEZ-DUGO et al. 2012). The above cause-and-effect relationships are often related to N utilization and usually account for the final stage of plant development. A better understanding of plant responses in different stages of growth and development will supplement the existing knowledge base because competitive interactions are already observed in the initial growth stages of plants and continue to occur, with varied intensity, until the end of the growing season (WANIC et al. 2013).

The aim of this study was to determine the effect of a sowing method of spring barley and Italian ryegrass (pure, mixture) and different water conditions on the nitrogen content and accumulation in the aerial biomass of plants at different stages.

MATERIAL AND METHODS

A pot experiment was conducted at the Greenhouse Lab of the Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn. Three experimental series were carried out: first – from 18 March to 30 June 2009, second – from 15 March to 05 July 2010, third – from 18 April to 22 July 2011. The tested plants were spring barley (hullless cv. Rastik) and Italian ryegrass (cv. Gaza).

The experimental factors were as follows:

- 1st order factor – sowing regime: pure-sown spring barley (pure sowing – *P*) and spring barley undersown with Italian ryegrass (mixed sowing – *M*);
- 2nd order factor – water supply: optimal water supply (*O*) and water supply reduced by 50% (*L*).

Soil material was obtained from the uppermost layer of proper brown soil developed from slightly loamy silty sand, characterized by slightly acidic pH, humus content of 1.22 - 1.91% and moderate levels of phosphorus, potassium and magnesium. One week before sowing, each pot was filled with 8 kg of soil which had been thoroughly mixed with mineral fertilizers (g pot⁻¹, pure ingredient basis): N – 0.5 (urea), P – 0.2 (monopotassium phosphate), K – 0.45 (potassium sulfate).

In treatments with optimal water conditions, water was supplied in the amount of 17 000 cm³ per pot during the growing season, and in treatments with reduced water supply – 8500 cm³. During the growing season, the amount of water supplied to plants differed depending on the growth stages of the analyzed species and soil moisture content.

The experiment had an additive design, according to which the number of plants in mixture was equal to the sum of crop densities in pure stands (SEMERE, FROUD-WILLIAMS 2001). 120 Kick-Brauckmann pots (with a diameter of 22 cm and a depth of 28 cm) were used in each experimental series: 3 sowing regimes (barley grown in pure stand, Italian ryegrass grown in pure stand, barley and Italian ryegrass grown in mixture x 2 levels of water supply x 5 growth stages x 4 replications). 18 spring barley or Italian ryegrass seeds able to germinate were sown in each pot (in mixture, 18 barley seeds and 18 Italian ryegrass seeds were sown), 3 cm deep. Using templates, seeds were spaced at equal distances from each other within the pot.

Ambient temperature in the laboratory was maintained at 20-22°C throughout the experiment, and it was lowered to 6-8°C for 9 days at full emergence to support vernalization.

The N content of the aerial biomass of plants was determined at five phenological development stages (BBCH-scale) of spring barley grown in pure stand under optimal soil moisture conditions: leaf development (10-13), tillering (22-25), stem elongation (33-37), heading (52-55) and ripening (87-91). When barley had reached each of the above stages, all plants were

removed from pots (used in a given growth stage) and the aerial parts were separated from the roots. The aerial parts of barley and ryegrass were divided into leaves, stems and spikes at successive growth stages. Plant material was air-dried, weighed and assayed for N content. All analyses were performed at the Chemical and Agricultural Station in Olsztyn (Standard PB 05). Nitrogen uptake was determined based on N content and biomass accumulation in plants (WANIC et al. 2013).

The mean values for three experimental series are presented in the tables. The data were analyzed statistically by analysis of variance (ANOVA). Homogeneous groups were identified by the Duncan's test. The probability of error was set at $p = 0.05$. The calculations were performed using the Statistica software.

RESULTS AND DISCUSSION

Nitrogen concentrations in the aerial biomass of barley varied throughout the growing season (Table 1). Nitrogen content was particularly high in leaves, and it decreased steadily during plant development. At the stem elongation and heading stages, the N content of leaves was still higher than in

Table 1

Nitrogen content of spring barley aerial biomass (g kg⁻¹ d.m.)

Growth stage (BBCH)	Plant part	Sowing regime		Water supply		Sowing regime			
		<i>P</i>	<i>M</i>	<i>O</i>	<i>L</i>	<i>P</i>		<i>M</i>	
						water supply			
		<i>O</i>	<i>L</i>	<i>O</i>	<i>L</i>	<i>O</i>	<i>L</i>	<i>O</i>	<i>L</i>
10-13*	leaves	49.6 <i>a</i>	51.2 <i>a</i>	50.1 <i>a</i>	50.7 <i>a</i>	49.4 <i>a</i>	49.7 <i>a</i>	50.7 <i>a</i>	51.7 <i>a</i>
22-25	leaves	44.5 <i>a</i>	44.5 <i>a</i>	44.3 <i>a</i>	44.7 <i>a</i>	44.2 <i>a</i>	44.8 <i>a</i>	44.3 <i>a</i>	44.6 <i>a</i>
33-37	leaves	41.3 <i>a</i>	41.1 <i>a</i>	41.9 <i>a</i>	40.6 <i>a</i>	42.1 <i>a</i>	40.6 <i>a</i>	41.7 <i>a</i>	40.5 <i>a</i>
	stems	27.4 <i>a</i>	26.7 <i>a</i>	26.9 <i>a</i>	27.3 <i>a</i>	27.1 <i>a</i>	27.7 <i>a</i>	26.6 <i>a</i>	26.8 <i>a</i>
52-55	leaves	29.4 <i>a</i>	29.0 <i>a</i>	28.1 <i>a</i>	30.3 <i>a</i>	28.5 <i>a</i>	30.2 <i>a</i>	27.6 <i>a</i>	30.4 <i>a</i>
	stems	19.4 <i>a</i>	18.5 <i>a</i>	19.3 <i>a</i>	18.6 <i>a</i>	19.5 <i>a</i>	19.3 <i>a</i>	19.1 <i>a</i>	17.9 <i>a</i>
	spikes	25.6 <i>a</i>	24.8 <i>a</i>	24.8 <i>a</i>	25.6 <i>a</i>	25.6 <i>a</i>	25.7 <i>a</i>	24.1 <i>a</i>	25.6 <i>a</i>
87-91	leaves	18.8 <i>a</i>	17.6 <i>a</i>	18.2 <i>a</i>	18.2 <i>a</i>	19.0 <i>a</i>	18.6 <i>a</i>	17.4 <i>a</i>	17.8 <i>a</i>
	stems	19.3 <i>a</i>	17.9 <i>a</i>	17.9 <i>a</i>	19.3 <i>a</i>	19.0 <i>a</i>	19.6 <i>a</i>	16.8 <i>a</i>	18.9 <i>a</i>
	spikes	30.0 <i>a</i>	27.3 <i>a</i>	28.0 <i>a</i>	29.4 <i>a</i>	29.3 <i>a</i>	30.8 <i>a</i>	26.6 <i>a</i>	28.0 <i>a</i>

* explanation in Material and Methods, *P* – pure-sown spring barley (pure sowing), *M* – spring barley undersown with Italian ryegrass (mixed sowing), *O* – optimal water supply, *L* – water supply reduced by 50%;

a – homogeneous groups (values followed by the same letters within experimental factors and their interactions are not significantly different at $p = 0.05$)

stems, but during the ripening N concentrations in stems and leaves were comparable. Nitrogen accumulation in spikes was the highest at the end of the growing season. Our results corroborate the findings of other authors (SOBKOWICZ 2003, DORDAS 2012, TIRYAKIOĞLU et al. 2014, WANIC et al. 2016). WOJCIESKA (1994) pointed to N “dilution” in plant tissues during rapid growth.

Neither the sowing regime nor the water supply exerted significant effects on the N content of the aerial biomass of spring barley (Table 1). However, some authors reported a relationship between N concentrations and precipitation levels during the growing season. In a study by WOJCIESKA (1994), nitrate concentrations in grass dry matter were lower in dry years than in wet years. GONZÁLEZ PONCE and SANTÍN MONTANYÁ (2007) demonstrated that the N content of winter barley grain and straw was significantly higher in a year with heavy rainfall. The effect of Italian ryegrass grown as undersown crop on the N content of barley grain has been documented in the literature. LEMOLA et al. (2000) found that N content was lower in spring barley mixed-sown with Italian ryegrass than in pure-sown spring barley.

Nitrogen uptake by the aerial biomass of spring barley was limited by the experimental factors: the presence of Italian ryegrass in the stand and water deficit (Table 2). It was only at the tillering stage, under optimal water supply, that the sowing regime had no significant effect on N accumulation in leaves. At the ripening stage, N uptake by barley stems was similar in

Table 2

Nitrogen uptake by spring barley (mg pot⁻¹)

Growth stage (BBCH)	Plant part	Sowing regime		Water supply		Sowing regime			
		<i>P</i>	<i>M</i>	<i>O</i>	<i>L</i>	<i>P</i>		<i>M</i>	
						<i>O</i>	<i>L</i>	<i>O</i>	<i>L</i>
		water supply							
10-13*	leaves	41.6 <i>a</i>	36.5 <i>b</i>	43.7 <i>a</i>	34.6 <i>b</i>	46.5 <i>a</i>	37.3 <i>c</i>	40.9 <i>b</i>	32.0 <i>d</i>
22-25	leaves	176.2 <i>a</i>	165.3 <i>b</i>	227.1 <i>a</i>	114.3 <i>b</i>	230.4 <i>a</i>	121.6 <i>b</i>	223.7 <i>a</i>	107.0 <i>c</i>
33-37	leaves	188.9 <i>a</i>	147.8 <i>b</i>	235.2 <i>a</i>	101.5 <i>b</i>	263.2 <i>a</i>	114.5 <i>c</i>	207.3 <i>b</i>	88.4 <i>d</i>
	stems	146.0 <i>a</i>	123.8 <i>b</i>	204.2 <i>a</i>	65.6 <i>b</i>	216.1 <i>a</i>	75.9 <i>c</i>	192.3 <i>b</i>	55.3 <i>d</i>
52-55	leaves	160.5 <i>a</i>	118.4 <i>b</i>	171.8 <i>a</i>	107.1 <i>b</i>	203.7 <i>a</i>	117.2 <i>c</i>	139.8 <i>b</i>	97.0 <i>d</i>
	stems	128.1 <i>a</i>	101.8 <i>b</i>	166.2 <i>a</i>	63.6 <i>b</i>	178.0 <i>a</i>	78.2 <i>c</i>	154.5 <i>b</i>	49.0 <i>d</i>
	spikes	49.2 <i>a</i>	34.6 <i>b</i>	64.8 <i>a</i>	19.0 <i>b</i>	74.3 <i>a</i>	24.0 <i>c</i>	55.2 <i>b</i>	14.1 <i>d</i>
87-91	leaves	82.1 <i>a</i>	70.5 <i>b</i>	91.5 <i>a</i>	61.1 <i>b</i>	97.6 <i>a</i>	66.6 <i>c</i>	85.5 <i>b</i>	55.5 <i>d</i>
	stems	118.0 <i>a</i>	91.5 <i>b</i>	116.6 <i>a</i>	92.9 <i>b</i>	126.8 <i>a</i>	109.1 <i>b</i>	106.3 <i>b</i>	76.7 <i>c</i>
	spikes	63.4 <i>a</i>	42.0 <i>b</i>	68.4 <i>a</i>	36.9 <i>b</i>	84.3 <i>a</i>	42.4 <i>c</i>	52.5 <i>b</i>	31.3 <i>d</i>

* explanation in Material and Methods, *P* – pure-sown spring barley (pure sowing), *M* – spring barley undersown with Italian ryegrass (mixed sowing), *O* – optimal water supply, *L* – water supply reduced by 50%;

a, b, c, d – homogeneous groups (values followed by the same letters within experimental factors and their interactions are not significantly different at $p = 0.05$)

pure stands under water deficit and in mixed stands when plants were supplied with water to meet their full requirements. The above results are related to lower biomass of barley grown in mixture with ryegrass and under water stress conditions (WANIC et al. 2013). Negative influence of ryegrass on the biomass development and N accumulation in barley has been well-documented in the literature. KÄNKÄNEN and ERIKSSON (2007) demonstrated that Italian ryegrass has a high competitive ability and considerably reduces the grain yield of spring barley when sown at high density. GALON et al. (2011) reported that the presence of *Lolium multiflorum* reduced tillering, leaf area and biomass accumulation in barley. GONZÁLEZ PONCE and SANTÍN MONTANYA (2007) also found that species of the genus *Lolium* reduced barley biomass, straw yield, grain yield, N uptake and the efficiency of N translocation to the grain. Other results were reported by THOMSEN (2005), who reported that the catch crop had no significant effect on the grain yield and N accumulation in the grain of the cover crop. According to LEMOLA et al. (2000), such a response is related to weak competition for light, water and macronutrients between mixture components in the early stages of slow growth. The water-stress tolerance of barley changes during its development; according to PECIO and WACH (2015), it is higher at the tillering stage than at the late boot stage (flag leaf sheath swollen).

The nitrogen content was higher in ryegrass leaves than in stems (Table 3). Nitrogen accumulation in the aerial biomass of ryegrass decreased in successive stages of development. According to GRYGIERZEC and RADKOWSKI (2006), this resulted from N “dilution” due to dry matter accumulation. The N con-

Table 3

Nitrogen content of Italian ryegrass aerial biomass (g kg⁻¹ d.m.)

Growth stage (BBCH)	Plant part	Sowing regime		Water supply		Sowing regime			
						<i>P</i>		<i>M</i>	
		<i>P</i>	<i>M</i>	<i>O</i>	<i>L</i>	water supply			
						<i>O</i>	<i>L</i>	<i>O</i>	<i>L</i>
10-13*	leaves	51.4 <i>a</i>	45.6 <i>a</i>	50.0 <i>a</i>	47.0 <i>a</i>	52.2 <i>a</i>	50.6 <i>a</i>	47.8 <i>a</i>	43.4 <i>a</i>
22-25	leaves	46.2 <i>a</i>	43.5 <i>b</i>	44.2 <i>b</i>	45.6 <i>a</i>	45.8 <i>ab</i>	46.6 <i>a</i>	42.5 <i>c</i>	44.6 <i>b</i>
33-37	leaves	43.2 <i>a</i>	40.2 <i>a</i>	41.1 <i>a</i>	42.3 <i>a</i>	42.3 <i>a</i>	44.1 <i>a</i>	39.9 <i>a</i>	40.5 <i>a</i>
	stems	31.4 <i>a</i>	27.5 <i>a</i>	29.8 <i>a</i>	29.1 <i>a</i>	30.2 <i>a</i>	32.6 <i>a</i>	29.3 <i>a</i>	25.6 <i>a</i>
52-55	leaves	31.4 <i>a</i>	30.3 <i>a</i>	29.6 <i>a</i>	32.1 <i>a</i>	29.5 <i>a</i>	33.2 <i>a</i>	29.6 <i>a</i>	31.0 <i>a</i>
	stems	18.1 <i>a</i>	19.6 <i>a</i>	20.3 <i>a</i>	17.4 <i>a</i>	18.7 <i>a</i>	17.5 <i>a</i>	21.9 <i>a</i>	17.2 <i>a</i>
87-91	leaves	23.1 <i>a</i>	23.8 <i>a</i>	21.4 <i>a</i>	25.5 <i>a</i>	21.4 <i>a</i>	24.7 <i>a</i>	21.3 <i>a</i>	26.3 <i>a</i>
	stems	15.5 <i>a</i>	15.9 <i>a</i>	13.1 <i>a</i>	18.4 <i>a</i>	12.8 <i>a</i>	18.3 <i>a</i>	13.4 <i>a</i>	18.5 <i>a</i>

* explanation in Material and Methods, *P* – pure-sown Italian ryegrass (pure sowing), *M* – Italian ryegrass as undersown crop for spring barley (mixed sowing), *O* – optimal water supply, *L* – water supply reduced by 50%;

a, *b* – homogeneous groups (values followed by the same letters within experimental factors and their interactions are not significantly different at $p = 0.05$)

tent of stems determined at the end of the growing season was comparable with the values reported by other authors (ZAJĄC, ANTONKIEWICZ 2006, PŁAZA et al. 2009, VRBNICANIN et al. 2012, BERNARDI et al. 2014). In the present study, the experimental factors affected N accumulation in shoots only at the tillering stage. Under the optimal water supply, mixed sowing with spring barley significantly reduced N concentrations in the aerial biomass of Italian ryegrass (Table 3). According to GRYGIERZEC and RADKOWSKI (2006), the content of protein and nitrogen in the dry matter of *Lolium multiflorum* and *Lolium x boucheanum* increases in years with abundant rainfall.

Nitrogen uptake by the dry matter of Italian ryegrass leaves and stems from emergence to ripening was significantly lower in mixed stands with spring barley than in pure stands (Table 4). The effect of water supply was

Table 4

Nitrogen uptake by Italian ryegrass (mg pot⁻¹)

Growth stage (BBCH)	Części rośliny	Sowing regime		Water supply		Sowing regime			
		P	M	O	L	P		M	
						water supply			
		O	L	O	L	O	L	O	L
10-13*	leaves	6.6a	5.6b	7.2a	5.0b	7.9a	5.2c	6.4b	4.7c
22-25	leaves	82.6a	33.5b	83.2a	32.9b	117.0a	48.2b	49.4b	17.7c
33-37	leaves	145.3a	56.9b	123.2a	79.1b	166.5a	124.1b	79.8c	34.0d
	stems	85.4a	17.3b	74.7a	27.9b	126.2a	44.5b	23.2c	11.3c
52-55	leaves	306.6a	92.1b	252.9a	145.8b	384.9a	228.2b	120.9c	63.4d
	stems	100.5a	32.4b	94.6a	38.3b	141.4a	59.7b	47.7c	17.0d
87-91	leaves	266.6a	101.9b	216.8a	151.7b	318.7a	214.6b	114.8c	88.9d
	stems	78.3a	29.7b	59.5a	48.5b	88.1a	68.5b	30.8c	28.6c

* explanations in material and Methods, P – pure-sown Italian ryegrass (pure sowing), M – Italian ryegrass as undersown crop for spring barley (mixed sowing), O – optimal water supply, L – water supply reduced by 50%;

a, b, c, d – homogeneous groups (values followed by the same letters within experimental factors and their interactions are not significantly different at $p = 0.05$)

also significant, namely the optimal water supply, compared with water supply reduced by 50%, contributed to a significantly higher N uptake by ryegrass biomass, noted throughout the growing season in pure stands. In mixed stands, during stem elongation and ripening, water supply had no influence on N uptake by stems. In the remaining phenological development stages, N uptake by aerial biomass (leaves and stems) was higher under optimal water supply than under water deficit. According to LEMOLA et al. (2000), N uptake is affected by soil type, but in our study N uptake was determined by competitive interactions between the analyzed species and water stress, which influenced biomass accumulation. WANIC et al. (2013) demonstrated that spring barley negatively affected Italian ryegrass development

from the beginning of coexistence of both species in mixed stands, whereas the response of ryegrass to the presence of barley was getting stronger until tillering. According to the authors, water stress exacerbated the adverse impact of mixed sowing. SIMIĆ et al. (2009) reported that *Lolium multiflorum* produced greater biomass and developed longer leaves and shoots under adequate moisture conditions. In a study by GONZÁLEZ PONCE and SANTÍN MONTANYÁ (2007), also *Lolium rigidum*, a weed species competing with winter barley, responded by increased biomass accumulation to intense rainfall. A decrease in biomass yield is a typical response to water stress, resulting from reduced photosynthetic rate (OLSZEWSKA et al. 2010, RUMASZ-RUDNICKA 2010, JASTRZĘBSKA et al. 2015).

CONCLUSIONS

1. Nitrogen concentrations in the aerial biomass of spring barley and Italian ryegrass varied throughout the growing season, and decreased steadily during plant development.

2. Neither the sowing regime nor the water supply exerted significant effects on the N content of the aerial biomass of spring barley. In spring barley, mixed sowing reduced N uptake by biomass, and the observed decrease was exacerbated under water deficit.

3. In Italian ryegrass, under optimal water supply, mixed sowing with spring barley decreased N concentrations in shoots only during tillering. Reduced water supply significantly decreased N accumulation in ryegrass biomass, but the effect of competitive interactions between the analyzed species on N uptake by aerial biomass was usually stronger than the influence of water deficit.

4. Differences in N uptake resulted from competitive interactions and water stress, which decreased aerial biomass accumulation in the companion crop and undersown crop.

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