

1 Nitrogen leaching and residual effect of barley/field bean 2 intercropping

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4 M. MARIOTTI^{1*}, A. MASONI², L. ERCOLI³, I. ARDUINI²

5
6 ¹ Department of Veterinary Science, University of Pisa, Pisa, Italy

7 ² Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

8 ³ Scuola Superiore Sant'Anna, Pisa, Italy

9 10 ABSTRACT

11 Cereal/legume intercropping may improve resource use efficiency in agroecosystems
12 and increase yield per unit surface and yield stability. Two field bean (*Vicia faba* L.)
13 and four barley (*Hordeum vulgare* L.) varieties were mono- and intercropped (additive
14 design) in a 2-year lysimeter experiment on a sandy loam soil. The aim was to test the
15 effect of the cropping system on dry matter and N yield of forage, the residual effect on
16 the subsequent ryegrass crop (*Lolium multiflorum* Lam. *westerwoldicum*), and NO₃-N
17 leaching in the rotation. Land equivalent ratios were 1.65 for dry matter and 1.67 for N
18 yield, indicating a clear advantage of the intercrop over sole crops. Both species
19 suffered from competition, especially in terms of N resource, but barley was less
20 affected. Nitrate leaching was lowest from intercrop. Preceding crop significantly
21 affected dry matter, N content and NO₃-N leaching of ryegrass. Field bean sole crop
22 gave highest benefits to ryegrass in terms of forage dry matter and N content, but also
23 highest NO₃-N leaching, followed by the intercrop and the barley sole crop. Barley/field

1 bean intercropping may be an effective strategy to reduce land requirements, N leaching
2 losses and fertiliser inputs, thereby increasing the sustainability of farming systems.

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4 Keywords: cereal/legume; crop rotation; mixture; N benefit; N loss

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9 Intercropping grass and legume is promoted to increase yield per unit surface and
10 yield stability (Strydhorst et al. 2008). In temperate climates, cereal-legume
11 intercropping has been more successful when used for forage than grain production
12 (Anil et al. 1998). Forage obtained from cereal/legume intercrops (IC) always has a
13 higher quality than that of cereal sole crops (SC), while dry matter yield advantage
14 varies, due to large differences in growth conditions (Dordas and Lithourgidis 2011).
15 Intercrop yield is generally between that of SC (Carr et al. 2004, Strydhorst et al. 2008),
16 or higher over both (Karpenstein-Machan and Stuelpnagel 2000, Ghanbari-Bonjar and
17 Lee 2003, Lithourgidis and Dordas 2010, Mariotti et al. 2012). According to Pursiainen
18 and Tuori (2008), also silage produced from a cereal/pulse legume mixture has a higher
19 nutritive value compared with that produced from only cereals, due to the higher
20 concentration of crude protein, a higher degradability of nutrients and a better balance
21 of protein and energy for rumen microbes.

22 Additional advantages of cereal/legume intercropping include i) the more efficient
23 exploitation of the N resource, due to the complementarity use of biological N₂ fixation
24 and soil mineral N by companion plants; ii) the reduction in leaching N losses; and iii) a

1 positive residual effect on the next crop (Hauggaard-Nielsen et al. 2003, Szumigalski
2 and Van Acker 2006, Pappa et al. 2012).

3 Limited research has been carried out on barley/field bean intercropping for forage
4 production (Strydhorst et al. 2008, Lithourgidis and Dordas 2010), and to the best of our
5 knowledge no one has studied its N leaching and residual effects. This is despite the fact
6 that both crops have broad environmental adaptability, almost simultaneously reach the
7 optimal growth stage for ensilage, and provide a high quality feed (Carr et al. 2004,
8 Köpke and Nemeček 2010).

9 We assessed the agronomic advantages of barley/field bean intercropping for silage
10 production, by investigating i) the dry matter and N yield of sole crops and intercrops;
11 ii) the residual effect on the subsequent ryegrass crop, and iii) NO₃-N leaching losses in
12 the rotation. Since the choice of cultivar is a key factor influencing interspecific
13 competition and the amount of N available to the system (Ross et al. 2004, Pappa et al.
14 2012), we compared the combination between four barley (two 2-row and two 6-row)
15 and two field bean varieties.

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17 MATERIALS AND METHODS

18 The research was carried out in 2007-2008 and 2008-2009 at the Department of
19 Agriculture, Food and Environment of the University of Pisa, Italy, which is located
20 approximately 10 km from the sea (43° 40' N, 10° 19' E) and 1 m above sea level. The
21 climate of the area is hot summer Mediterranean, with mean annual daily air
22 temperatures of 14.2 °C and a mean annual rainfall of 971 mm. During both growing
23 seasons, daily minimum and maximum temperatures and rainfall were obtained from a
24 meteorological station located within 100 m of the trial site.

1 Plants were grown in an open-air facility consisting of 42 lysimeters of 300-L
2 volume (0.5 m² area and 0.6 m depth), spaced 20 cm apart, and embedded in expanded
3 clay to avoid daily fluctuations in soil temperature. For leachate collection, lysimeters
4 were attached to a 3 cm rigid PVC drain ending in a 30-L PVC tank. Approximately six
5 months before seeding, lysimeters were filled with soil collected from a field previously
6 cultivated with rapeseed (*Brassica napus* L.). Soil properties were: 56.8% sand (2 mm >
7 \varnothing > 0.05 mm), 27.5% silt (0.05 m > \varnothing > 0.002 mm), 15.7% clay (\varnothing < 0.002 mm), 7.9
8 pH (H₂O), 2.1% organic matter (Walkley and Black method), 1.0 g/kg total N (Kjeldahl
9 method), 22.0 mg/kg available P (Olsen method), 72.4 mg/kg available K (BaCl₂-TEA
10 method), and 8.4% total CaCO₃ (Scheibler method).

11 In 2007-2008, experimental treatments consisted of two 2-row barley varieties
12 (Naturel and Ninfa), two 6-row barley varieties (Gotic and Sonora), two field bean
13 varieties (Chiaro di Torrelama and Vesuvio), and eight intercrops obtained by the
14 combination of each barley and field bean variety. In total there were 14 crop treatments
15 arranged in a randomized block design with three replicates. The 2-row barley cv. have
16 more leaves per culm, more spikes per unit area and a higher mean kernel weight, but
17 fewer grains per spike than 6-row cv. Field bean Chiaro di Torrelama is more cold
18 resistant and taller than Vesuvio.

19 Barley and field bean were sown on 9 November 2007. The seeding rate was 400
20 seeds m⁻² with a 16-cm row spacing for barley, and 50 seeds m⁻² with a 32-cm row
21 spacing for field bean. Intercropping followed a 1:1 additive design, i.e. the density of
22 each component was the same as the density of the sole crop (Figure 1). All SCs and
23 ICs were fertilised pre-planting with urea, triple mineral phosphate and potassium
24 sulphate, at rates of 15 kg/ha of N, 65 kg/ha of P, and 125 kg/ha of K.

1 Sole crops and intercrops were harvested for silage at the dough-ripening stage of
2 barley (9 May 2008), when field bean was at the beginning of the pod development
3 stage. In June 2008, crop residues were incorporated into the soil and, in order to
4 estimate their residual effect, ryegrass was grown in all lysimeters. On 6 October 2008,
5 ryegrass (cv. Jivet) was sown at a rate of 300 seeds/m² with a 16-cm row spacing. It was
6 fertilised pre-planting with triple mineral phosphate and potassium sulphate, at a rate of
7 30 kg/ha of P and 60 kg/ha of K. No N fertiliser was applied. Ryegrass was harvested at
8 the stage of spike emergence (14 May 2009).

9 Plants of all crops were cut 5 cm above ground level and intercrops were separated
10 into cereal and legume. Ryegrass roots were separated from the soil by gently washing
11 with water. Plant parts were oven-dried at 75 °C to constant weight for determination of
12 dry matter, and analysed for N concentration by the micro Kjeldahl method. Nitrogen
13 yield was obtained by multiplying N concentration by DM. Throughout the study,
14 drainage water was collected from each lysimeter and analysed for volume and NO₃-N
15 concentration (Orion ion analyzer).

16 The advantage in DM and N yield of barley/field bean IC compared to SCs was
17 evaluated by the land equivalent ratio (LER), obtained as the sum of partial LERs
18 (Trydeman-Knudsen et al. 2004). Partial LER of barley (LER_b) and field bean (LER_{fb})
19 were calculated as the ratio between the DM, or N, yield in ICs and SCs. The
20 competitive ability of the two companion crops was estimated using the competitive
21 balance index (C_b) as $C_b = \ln (LER_b / LER_{fb})$ (Wilson 1988).

22 Field bean symbiotic N₂ fixation was estimated using the N-difference method
23 (Trydeman-Knudsen et al. 2004) as $N_2 \text{ fixed}_{fb SC} = N_{fb SC} - N_{barley SC}$, and the amounts of

1 N₂ fixed in intercropped field bean were calculated as: $N_2 \text{ fixed}_{fb \text{ IC}} = (N_{fb \text{ IC}} + N_{\text{barley IC}}) -$
2 $N_{\text{barley SC}}$.

3 Analysis of variance (ANOVA) was performed using CoStat 6.4 (CoHort Software,
4 CA, USA). Barley proportion in IC, LER and C_b data were analysed using a randomised
5 block design with eight treatments. For all other characters, ANOVA was carried out
6 using a randomised block design with 14 treatments. Significantly different means were
7 separated at $P < 0.05$ by the least significant difference (LSD) test (Steel et al. 1997).

8

9 RESULTS AND DISCUSSION

10 The four barley and the two field bean varieties did not differ statistically in terms of
11 DM and N yield in SC and IC, as well as in terms of residual effect and N leaching.
12 Accordingly, species mean effects only are reported.

13 The barley/field bean additive IC yielded 14 t/ha of forage DM, which was 100%
14 and 40% higher than the yield of barley and field bean SCs, respectively (Figure 2). The
15 LER for DM showed that resources were used 65% more efficiently in IC, while partial
16 LERs (0.87 for barley and 0.78 for field bean) indicated that both species suffered from
17 competition, with field bean being more affected. Although we did not examine the
18 quality of the forage mixture, the proportion of barley was 45%, falling within the range
19 of 25-50% recommended for cereal/legume silage (Pursiainen and Tuori 2008).

20 Intercropping did not affect the N concentration of companion species and gave an N
21 yield of 316 kg/ha (Figure 2). Thus, the N yield of IC equalled that of field bean SC and
22 was three times higher than barley SC. The LER showed that N was used 67% more
23 efficiently in IC, while partial LERs (0.90 for barley and 0.77 for field bean) highlighted
24 that field bean had suffered the most also in terms of N uptake. Since the N yield of

1 barley was lower in IC than SC, and N concentration was not affected, we suggest that
2 there was no N-transfer from field bean to barley.

3 The results of the competitive balance index agreed with those of partial LERs,
4 indicating that barley dominated over field bean in the IC. In addition, the higher C_b
5 index for N (0.16) than for DM yield (0.11) suggests that barley was more competitive
6 for N than for other resources (light, water). Field bean fixed the same amount of N in
7 IC and SC (233 kg/ha) from which we suppose that biological N_2 fixation was not
8 affected by competition with barley (Dhima et al. 2007). In contrast, IC significantly
9 reduced mineral N uptake of field bean, which accounted for only a 3% N content in IC
10 compared to 26% in SC. The increased proportion of N derived from N_2 fixation in
11 legumes intercropped with cereals suggests that root competition was greater than shoot
12 competition (Martin and Snaydon 1982, Hauggaard-Nielsen et al. 2003, Trydeman-
13 Knudsen et al. 2004). The higher competitiveness of grass roots is likely due to their
14 faster growth in autumn-winter and to their finer and deeper root system, allowing a
15 more efficient exploitation of the soil volume and a higher nutrient uptake (Hauggaard-
16 Nielsen et al. 2009, Dordas and Lithourgidis 2011). In conditions that enable the growth
17 of both companion species, the NO_3 -N uptake of the grass causes a temporary decrease
18 in its amount in soil, increasing the proportion of fixed N_2 of the legume (Opitz von
19 Boberfeld et al. 2005, Szumigalski and Van Acker 2006).

20 The amount of water drained throughout the entire growing season (Figure 3) was
21 slightly lower in IC (271 mm) than SCs (approximately 295 mm) and represented 53-
22 57% of rainfall (514 mm). Nitrate concentration in leachates was highest in November-
23 December, averaging 34 mg/L without appreciable differences among crop systems.
24 Values decreased markedly in January and fell close to zero in February for barley and

1 IC, and two months later for field bean (Figure 3). Total NO₃-N leaching was lowest
2 from IC (34 kg/ha), with a minor loss of 22 kg/ha compared to field bean SC, and 7
3 kg/ha compared to barley SC. Nitrate leaching in December accounted for 49% of total
4 NO₃-N losses in field bean and 64% in barley and IC. After February, NO₃-N lost by
5 leaching was negligible in all crop systems, due to reduced rainfall and increased crop
6 evapotranspiration. We believe that the lower water drainage and NO₃-N leaching from
7 IC were primarily due to higher plant density. However, Hauggaard-Nielsen et al.
8 (2001) reported that IC induced a faster lateral root development in both pea and barley
9 and a deeper growing root system in the cereal, which could also contribute to reducing
10 leaching.

11 The DM yield of ryegrass forage was significantly affected by preceding crop
12 (Figure 4). The greatest yield was observed after field bean SC, which was 62% higher
13 than after barley SC, but only 17% higher than after IC. Nitrogen concentration was not
14 affected by preceding crops (results not shown); thus also the N content of ryegrass
15 forage after field bean was 55% higher than after barley and 12% than after IC (Figure
16 4). Similar rankings were observed for residuals (roots+stubbles), whose ratios to forage
17 were approximately 3.7 for DM and 5.1 for N content, without appreciable differences
18 due to the preceding crop.

19 No leaching occurred from the harvest of barley and field bean SC and IC to the
20 sowing of ryegrass. During ryegrass growth, drainage volume was not affected by
21 preceding crops while the amount of NO₃-N leached was 31 kg/ha after field bean SC,
22 27 kg/ha after IC, and 21 kg/ha after barley SC (Figure 5). From 89% to 98% of total N
23 was leached in November, with a flow-weighted N concentration of approximately 24

1 mg/L following field bean and IC, and 19 mg/L following barley. After November
2 values were lower than 1 mg/L.

3 Residual effect and N leaching of the following crop are both related to the N content
4 and decomposition rate of preceding crop residues. Compared to cereal residues, those
5 of grain legumes are higher in N content and lower in C/N ratio, which results in fast
6 and high N mineralization and accumulation of NO₃-N in soil, available either for
7 subsequent crop uptake or leaching (Karpenstein-Machan and Stuelpnagel 2000). This
8 explains both the higher residual effect and NO₃-N leaching after field bean than after
9 barley SCs. Intermediate values obtained after IC may be due to a slower net N
10 mineralization during residue decomposition, because N released from field bean
11 residues may be immobilised by those of the companion barley that have a greater C/N
12 ratio (Hauggaard-Nielsen et al. 2003). The more balanced chemical composition of IC
13 residues may result in less net N mineralization in autumn-winter leading to a lower N
14 leaching compared to field bean SC, and a higher residual effect compared to barley SC.

15 In conclusion, compared to SCs, additive barley/field bean IC increased forage yield,
16 maintained a good quality for ensilage, and reduced NO₃-N leaching. Residual effects
17 of IC were intermediate between those of field bean and barley SCs in terms of yield
18 and N benefits, and N leaching to subsequent ryegrass. The choice of variety, either for
19 barley or field bean, did not affect significantly crop performance in SC and IC.
20 Barley/field bean IC can reduce land requirements, N leaching losses and fertiliser
21 inputs, thus complying with several goals of sustainable cropping.

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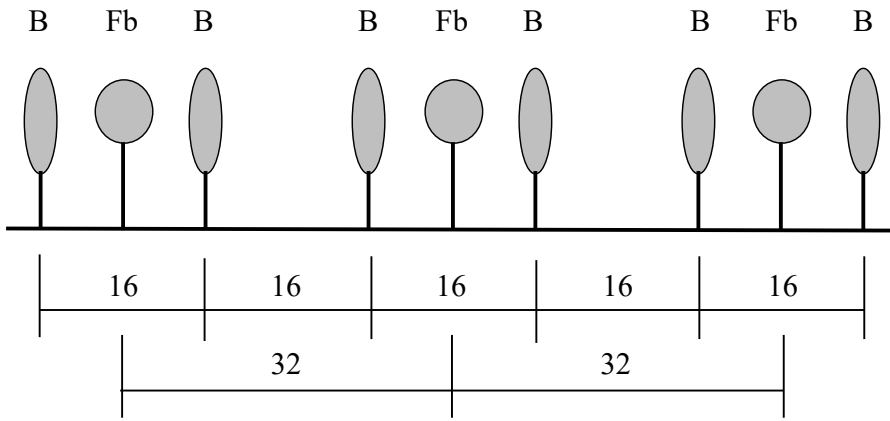
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15 Corresponding author:
16 Prof. Marco Mariotti, Department of Veterinary Science, University of Pisa, Via delle
17 Piagge 2, 56124 Pisa, Italy
18 e-mail: marco.mariotti@unipi.it
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1 Figure 1. Crop distribution and row spacing (cm) in IC.

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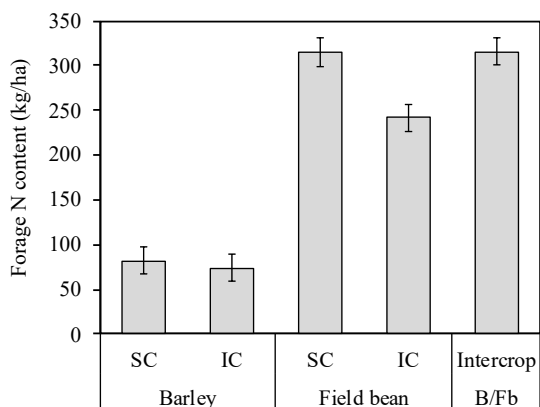
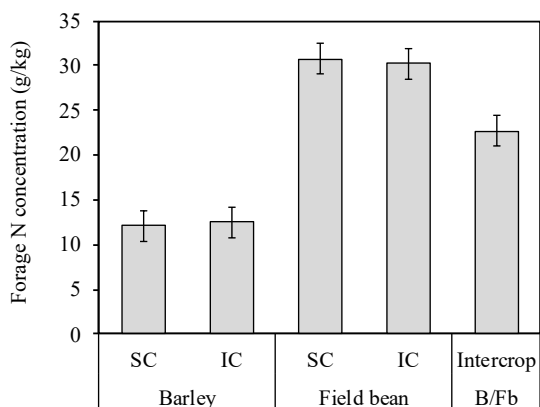
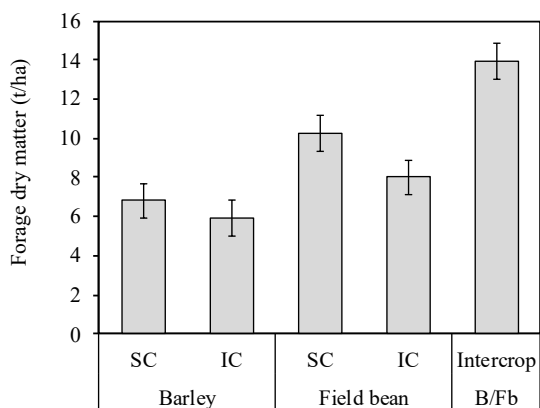


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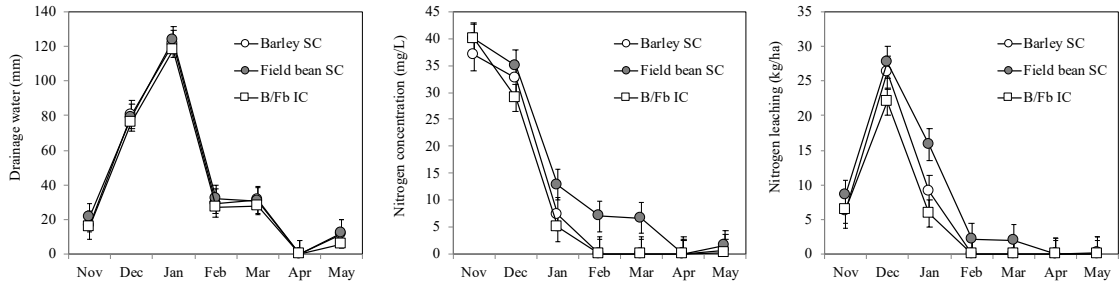
1 Figure 2. Forage dry matter, N concentration and content of barley and field bean sole
 2 crops and intercrops. In this and following figures, vertical bars denote LSD; when not
 3 visible, bars lie within the symbol.
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1 Figure 3. Water drainage, NO₃-N concentration in leachate, and NO₃-N leached by
 2 barley and field bean sole crops and intercrops.

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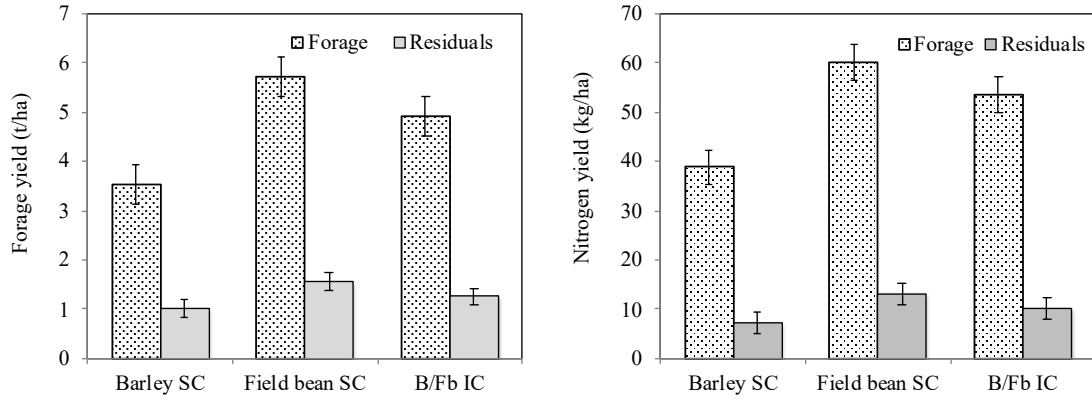
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1 Figure 4. Dry matter and N content of ryegrass forage and residuals (roots+stubbles).

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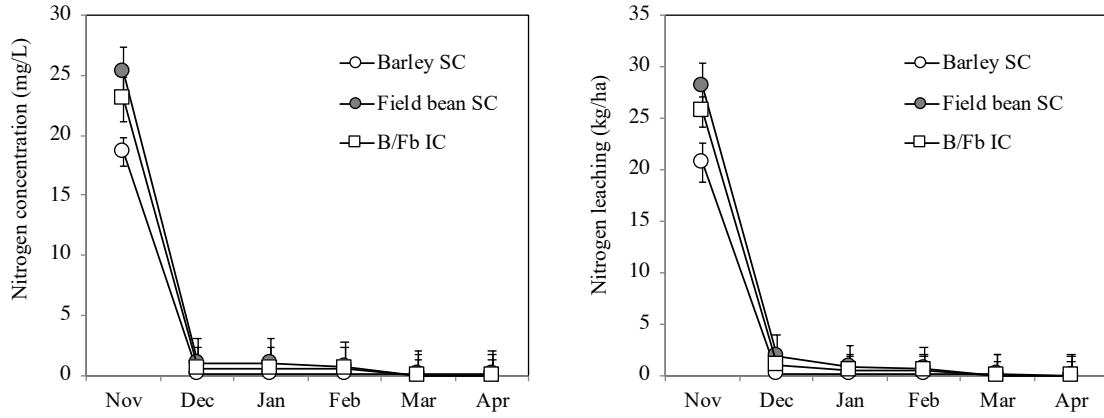
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1 Figure 5. Nitrate concentration in leachate and NO₃-N leached by ryegrass.

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