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Biosolids affect the growth, nitrogen accumulation and nitrogen leaching of barley

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ABSTRACT

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Biosolids are organic fertilisers derived from treated and stabilised sewage sludge that increase soil fertility and supply nitrogen to crops over a long period, but can also increase the risk of nitrogen (N) leaching. In this work, spring barley was grown in lysimeters filled with soil amended with biosolids, and with and without mineral N fertilisation. Biomass and the N concentration and content of shoots and roots were determined at flowering and maturity, and the N remobilization was calculated during grain filling. Drainage water was collected and analysed for N leaching. Biosolids increased soil porosity and soil nitrate, and positively affected the growth and N uptake of barley. Compared to mineral fertilisers, biosolids produced 18% higher vegetative biomass and 40% higher grain yield. During grain filling, both N uptake and N remobilization were higher with biosolids, which increased the grain N content by 32%. Nitrogen loss in leachates was 1.2% of plant uptake with mineral fertilisers and 1.7% with biosolids. Thus, soil fertilisation with biosolids greatly benefits spring barley, only slightly increasing N leaching.

Keywords: cereals; *Hordeum vulgare*; N nutrition; organic wastewater solids; yield components

Biosolids are organic fertilisers derived from treated and stabilised sewage sludge, which meet the pollutant and pathogen requirements for agriculture application, and are rich in organic matter and nutrients, particularly nitrogen and phosphorous (Binder et al. 2002). Applying biosolids at agronomic rates was found to positively affect soil fertility and growth of several crops, and to produce equivalent or greater yields than inorganic fertilisers (Christie et al. 2001, Sullivan et al. 2009).

Most nitrogen (N) in biosolids is contained in the organic matter and only small amounts are present as available nitrate and ammonium (Esperschuetz et al. 2016b, Rigby et al. 2016). Nitrogen locked up in organic compounds is released slowly throughout the crop cycle and it thus nourishes the plants at a slow rate over a long period, more closely matching crop requirements than inorganic fertilisers (Eldridge et al. 2008). Since in cereals, N accumulation in the grain originates from the current uptake transferred directly to kernels and from the remobilisation of N stored temporarily in vegetative plant parts (Masoni et al. 2007), higher N-availability in soil during grain filling could change the proportion of the two fractions. Increased N-uptake in biosolids-amended plants has been reported (Sharma et al. 2017), but few experimental data regarding the effect of biosolids on N uptake and remobilisation during grain-filling are available (Koutroubas et al. 2014).

When mineralised-N from biosolids exceeds crop uptake, a surplus NO_3^--N is released into the soil, which may cause excessive N-leaching to groundwater (Binder et al. 2002). The timing of biosolids application is thus crucial to minimize leaching and, in the Mediterranean region where

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the rainfall is concentrated between November–February, their distribution in late fall can result in greater N losses compared to late winter or spring distributions (Fumagalli et al. 2013). The application of biosolids to cool-season cereals is thus discouraged or banned. The risk of NO_3^- leaching may be reduced by distributing biosolids at the end of winter to the benefit of spring cereals, because lower rainfall and higher evapotranspiration after winter markedly reduce drainage water.

On the basis that biosolids increase N uptake during grain filling, the aim of the present research was to assess the effects of the application of biosolids and mineral N fertilisers to soil, on the growth, yield, and nitrogen dynamics (soil uptake, remobilisation to grain, and leaching) of spring barley grown in a Mediterranean environment.

MATERIAL AND METHODS

Site description and experimental design. The research was carried out in 2015 and 2016 at the Research Centre of the Department of Agriculture, Food and Environment of the University of Pisa, Italy, located at a distance of approximately 4 km from the sea (43°40'N, 10°19'E) and 1 m a.s.l.

The six-row barley (*Hordeum vulgare* L.) cv. Mattina was used. In both years, experimental treatments consisted of three fertiliser treatments and two harvest times, arranged in a split-plot design with three replications. The fertiliser treatments consisted of a control with no N fertilisation (C), mineral N fertilisation (MF) applied at 120 kg N/ha (0.6 g N/lysimeter), and biosolids (B) applied at 20 t/ha dry weight (98 g/lysimeter). The MF rate is that recommended for spring barley in Central Italy and the rate of B corresponds to the highest rate that can be applied to agricultural soils in Italy. Harvest times were flowering (26 May 2015 and 18 May 2016) and maturity (1 July 2015 and 28 June 2016). Part of the inorganic N fertiliser (20 kg N/ha) was incorporated in the soil just before sowing as $(NH_4)_2\,SO_4$. The remaining amount (100 kg N/ha) was top-dressed as urea at the start of stem elongation (16 April 2015 and 20 April 2016). Anaerobically digested and dewatered biosolids (Table 1), obtained from the wastewater treatment plant of Livorno (Italy), were mixed with the soil before lysimeter filling. Phosphorus and potassium were applied pre-planting as triple superphosphate and potassium sulphate, at the rate of 33 kg P/ha and 62 kg K/ha.

Experimental equipment and crop management. The research was carried out in an open-air facility consisting of 18 small lysimeter tubes made from polyvinyl chloride (60 cm long by 25 cm diameter) filled with 35 kg of soil. To measure NO_3^--N and NH_4^+-N leaching, a drainage-sampling device was installed at the bottom of each lysimeter. In both years, lysimeters were filled with a sandy-loam soil collected from a field previously cultivated with oilseed rape. Soil properties were similar for the two years of the trial, and average values were: 54.9% sand (2–0.05 mm), 33.5% silt (0.05–0.002 mm), 11.6% clay (< 0.002 mm), 7.6 pH (H_2O), 0.7 g/kg total nitrogen (Kjeldahl method), 4.4 mg/kg available P (Olsen method), and 69.3 mg/kg available K (ammonium acetate).

Barley was sown on 3 March 2015 and 2 March 2016. After emergence, the seedlings were thinned to 20 plants per lysimeter. Weed control was performed by hand. In 2015, plants were irrigated with drinking water from flowering to maturity to prevent water stress. In 2016, irrigation was not performed because of sufficient rainfall.

Weather conditions. Minimum and maximum air temperatures and rainfall were obtained from a meteorological station close to the experimental site. Total rainfall of the growth season was 152 mm in 2015 and 430 mm in 2016 (Figure 1).

Table 1. Selected properties of biosolids (dry weight basis)

Biosolids properties		Value
pH		6.4
Total organic C		38.5
Total N	(%)	7.9
Total P		1.2
Humification degree		1.9
Total phenolic compounds	(g/kg)	0.6
Cr^{VI}		< 1
As		< 5.0
Cd		< 2.0
Cr^{III}		16
Hg	(mg/kg)	< 0.1
Ni		25
Pb		12.5
Cu		72.4
Zn		185.1

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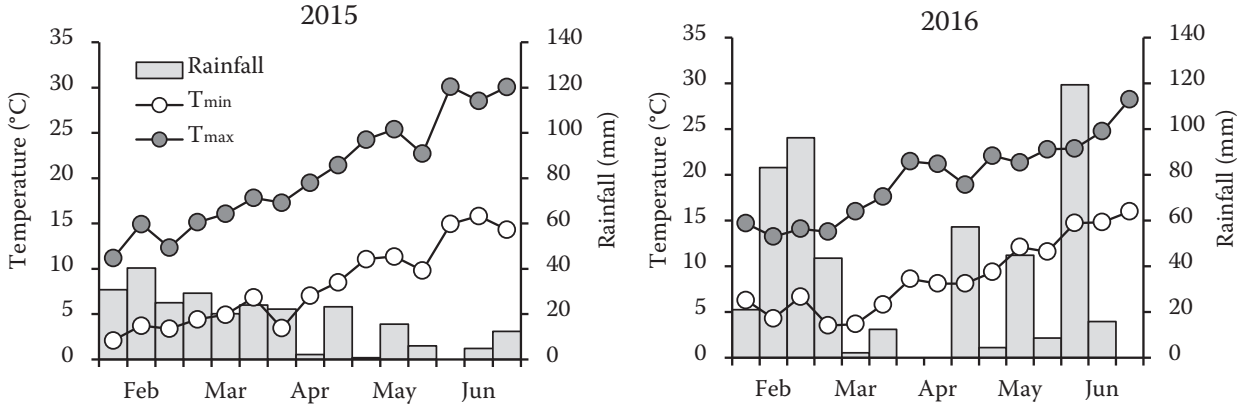

Figure 1. Air minimum and maximum temperatures and rainfall in the two growth seasons

Temperatures were similar in both years, ranging from –0.3°C to 28.7°C.

Data collection. The pH, nitrate concentration, bulk density, and porosity of soil were determined for each lysimeter before sowing, at the stages of 1st node detectable, flowering, and maturity. Soil nitrate concentration was determined by the ion chromatography (Dionex apparatus, mod. DX-100, Sunnyvale, USA). Soil bulk density was determined by the core method, i.e. weighing the undisturbed soil samples provided by a cylindrical core (4 cm diameter and 5 cm height). Soil particle density was determined by a pycnometer. Total soil porosity was calculated as:

$$1 - (\text{soil bulk density}/\text{soil particle density}) \times 100.$$

At each harvest, plants were manually cut at the ground level and the shoots were separated into culms + leaves and spikes. Dead leaves were also collected. Spikes were counted and, at maturity, separated into kernels and chaff. Roots were recovered from the soil by gently washing with a low flow from sprinklers and then separated from the base of culms that were added to the aboveground part. The dry weight of all plant parts was determined by oven-drying at 60°C to constant weight, and samples were analysed for N concentration. Nitrogen content was obtained by multiplying N concentrations by DW. Mean kernel weight was determined. Drainage water was collected throughout the entire growth period. Leachate volumes were measured and their NH_4^+-N and NO_3^--N concentrations were determined with an Orion ion analyser, model 502A (Orion Research Inc., Boston, USA). Assuming that all N lost from vegetative parts between flowering and maturity was remobilised to the grain, the N apparent remobilisation was calculated as the difference between the N content of shoots or roots at flowering and the N content of culms + leaves + chaff or roots at maturity (Masoni et al. 2007).

Statistical analysis. Results were subjected to ANOVA. The effects of year, fertiliser treatment and harvest time and their interactions were analysed using a split-split-plot design. Grain yield, grain yield components and grain N concentration and content were analysed using a split-plot design. Significantly different means were separated at the 0.05 probability level by the Tukey's test. Analysis of variance revealed that neither the main year effect nor the year interactions affected any of the parameters measured, probably because the differences in temperature between the two years were negligible and the crops were irrigated when necessary.

RESULTS AND DISCUSSION

Soil properties. Biosolids significantly reduced soil bulk density and increased soil porosity at flowering and maturity (Figure 2). This was primarily attributed to the organic matter that binds flocculating soil particles to form stable aggregates, thus improving soil structure and increasing total volume of macro- and micro pores (Mariscal-Sancho et al. 2011). Cardelli et al. (2017) also reported an increased biological activity. The variation in soil pH was negligible (data not shown).

After sowing, soil nitrate peaked in MF in response to the top-dress application and decreased progressively in B and C. However, both at flowering and maturity, levels were higher in B than in other treatments (Figure 2). At maturity, the NO_3^--N

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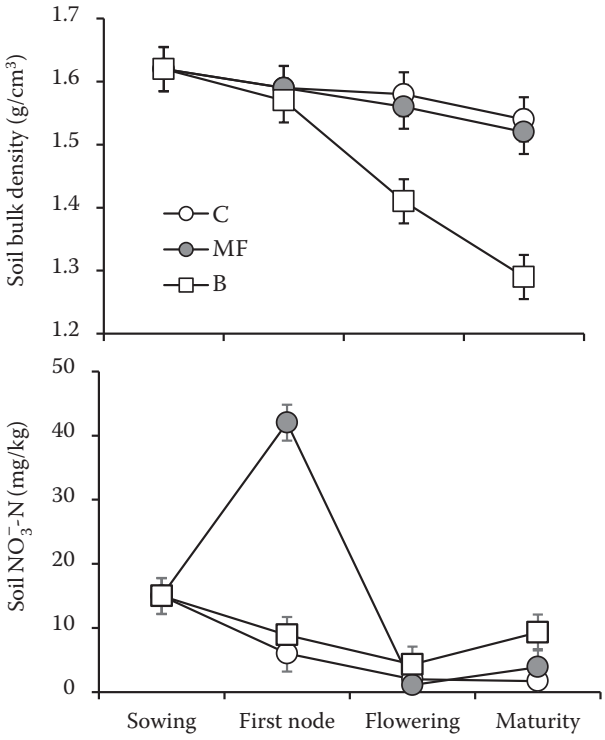

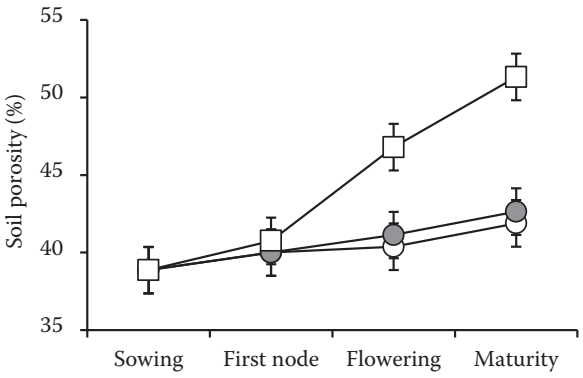

Figure 2. Bulk density, porosity and nitrate concentration of the 5-cm soil top layer, as affected by fertiliser treatment and sampling time. Vertical bars denote *HSD* (honest significant difference) at $P \leq 0.05$. C – no nitrogen fertilisation; MF – mineral nitrogen fertilisation; B – biosolids

concentration in the B-amended soil was similar or lower than at sowing, suggesting that most N released from biosolids was taken up by plants, greatly reducing leaching risk (Luo et al. 2003).

Plant growth, grain yield and N content. At both flowering and maturity, the application of biosolids to soil increased the dry weight of the vegetative aboveground part and of the roots compared to MF and C (Figure 3). The overall gain in vegetative biomass of B on MF was approximately 18% at both stages, and was primarily due to the development of more fertile tillers.

The N concentration of vegetative aboveground parts (0.7%) was not affected by fertilisation, whereas that of roots was higher in B and MF than in C (1.2% vs. 0.8%) at flowering, and did not vary among treatments (0.7%) at maturity (data not shown). The N accumulation in the vegetative aboveground part followed a pattern similar to the dry matter (Figure 3): it was 17% higher in B than MF and about 7-fold higher in B than C at both stages. Conversely, the N content of roots was 29% higher at flowering and 69% higher at maturity in B compared to MF, and 4.5- and 6.5-fold higher in B than in C plants. Higher N uptake and concentration in response to biosolids application is known (Koutroubas et al. 2014, Esperscheutz et al. 2016b), but the role of roots in belowground N storage had never been revealed.

Grain yield was highest with the B amendment, due to the higher numbers of spikes per plant and kernels per spike, whereas the mean kernel weight was not affected (Table 2). The grain N concentrations of B and MF-amended plants were similar and higher than C (Table 2). As a result, the grain N content of B-amended plants was 32% higher than MF, and 7-fold higher than C. At flowering, the overall N uptake per lysimeter was 223 mg in C, 1140 mg in MF, and 1362 mg in B treatments. From flowering to maturity the N uptake was 146, 701, and 1028 mg, respectively, corresponding to approximately 40% of total N uptake in all treatments. The above figures confirm our hypothesis that soils amended with biosolids improve N uptake throughout the entire growth cycle.

The higher growth, grain yield and nitrogen uptake of B compared to MF-fertilised plants highlight that barley is sensitive to the different amounts of available N and the different patterns of N release of the two fertilisers. According to US EPA, in temperate regions, the mineralisation factor of biosolids in the first year is 20% of the organic N fraction (Rigby et al. 2016). Accordingly, it was estimated that the distribution of 20 t/ha biosolids made approximately 100–125 kg/ha of potentially mineralisable N available for plant uptake in the 4-month growing season of spring barley. This

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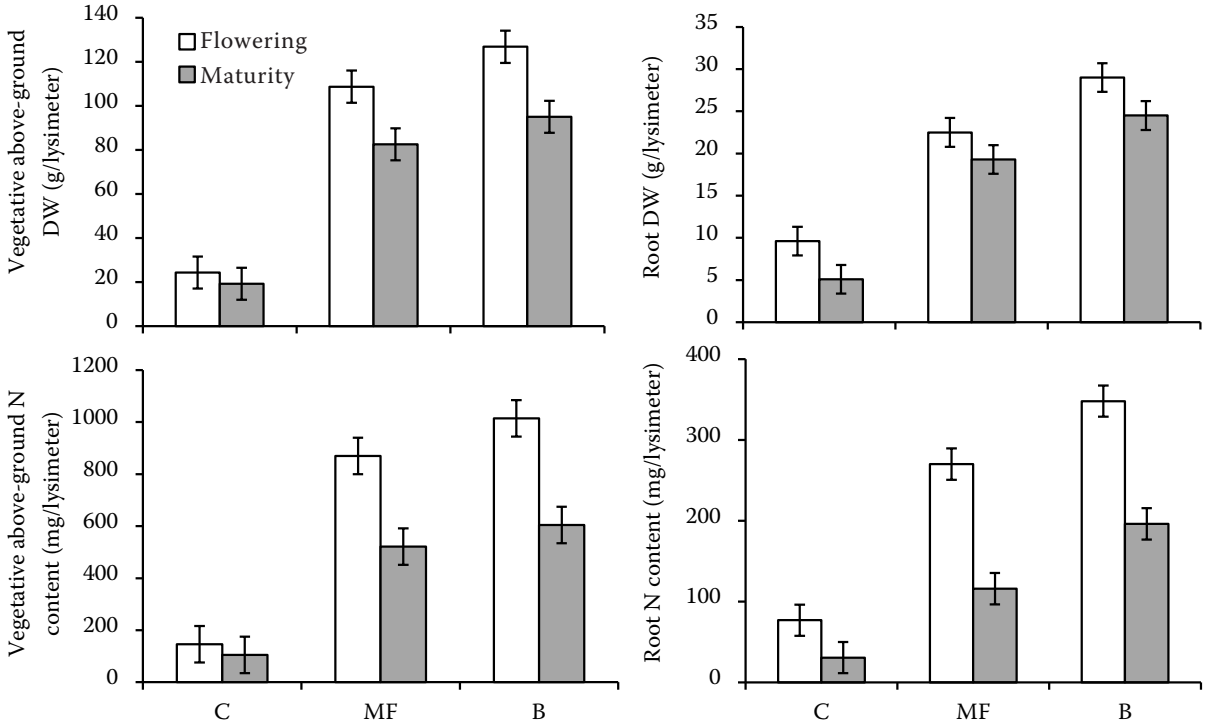

Figure 3. Dry weight and nitrogen (N) content of the aboveground vegetative part and of roots, as affected by fertiliser treatment and harvest time. Vertical bars denote *HSD* (honest significant difference) at $P \leq 0.05$. DW – dry weight; C – no nitrogen fertilisation; MF – mineral nitrogen fertilisation; B – biosolids

amount was close to the MF rate, but produced 32% higher grain N, due to the combination of a more constant release of N throughout the growth cycle, and the improvement of soil physical properties, primarily porosity (Kępka et al. 2016).

The beneficial effects of biosolids application on the growth and N uptake of spring barley may also be attributed to the supply of micronutrients (Esperschuetz et al. 2016a), and to the improvement of soil biological activity (Elbl et al. 2014, Plošek et al. 2017). Thus, biosolids provide more benefits than mineral fertilisers, especially in semiarid conditions and in soils that are low in organic matter, which is a common feature in the Mediterranean region (Antolin et al. 2005, Antoniadis et al. 2015).

Nitrogen dynamics during grain filling. In barley, the N requirement of growing kernels is fulfilled by the remobilisation of N assimilated before anthesis and by the current N uptake from soil. According to Przulj and Momcilovic (2001), the proportion of grain N derived from remobilisation ranges from 10% to 100%, and a surplus of soil N during grain filling favours N uptake and reduces remobilisation.

The N content of shoots roots always decreased between flowering and maturity, highlighting that all plant parts remobilised N to the grain with all treatments (Figure 4). However, the N remobilised from the shoot was 18% higher in B-amended plants than in MF, and 10-fold higher

Table 2. Grain yield, nitrogen (N) concentration and content, and grain yield components as affected by fertiliser treatments

Fertiliser treatment	Grain yield		Spike number	MKW	Kernel number	Grain N	
	(g/spike)	(g/lysimeter)	(*n*/plant)	(mg)	(*n*/spike)	(%)	(mg/lysimeter)
Control	0.6[a]	17.9[a]	1.6[a]	37.4[a]	15.3[a]	1.3[a]	232.7[a]
Mineral fertilisation	0.9[b]	75.2[b]	4.1[b]	43.4[b]	21.4[b]	1.6[b]	1203.2[b]
Biosolids	1.1[c]	106.0[c]	4.9[c]	45.6[b]	24.0[c]	1.5[b]	1590.0[c]

MKW – mean kernel weight. Values followed by different letters within a column differ at $P \leq 0.05$

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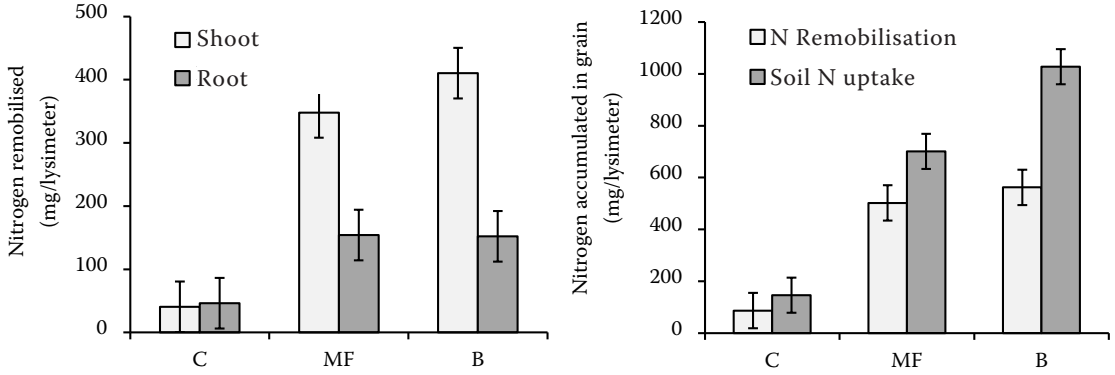

Figure 4. Nitrogen (N) remobilised by shoot and roots, and origin of grain N, as affected by fertiliser treatment. Vertical bars denote *HSD* (honest significant difference) at $P \leq 0.05$. C – no nitrogen fertilisation; MF – mineral nitrogen fertilisation; B – biosolids

than C plants, while N remobilised from roots was similar in B and MF plants and 3-fold lower in controls (Figure 4). Thus, in B and MF, shoots contributed to the total remobilisation by at least 70%, while in C plants shoots and roots remobilised about the same amount of N. Current N uptake was 1.5-fold and 7-fold higher with B application than with MF and C, respectively.

In all treatments, a higher proportion of grain N was derived from soil uptake, however percentages were higher in B (70%) than in C (63%) and MF (57%). Our results highlight that in the B treatment, the higher N available in soil during grain-filling reduced the proportion of root-N that was remobilized, which, however did not affect total remobilization, since the amounts of grain-N from both sources were higher than in MF and C.

Nitrogen leaching. Leaching was not observed in the first year because rainfall was never sufficient to cause percolation. In the second year, there were four leaching events, and the cumulative drainage was approximately 3.2 L/lysimeter, without differences among treatments (Figure 5).

In two March leachates, the N concentration of drainage water was lower than 10 mg/L without differences due to treatment, while in the leachates of May and June, B and MF application substantially increased the N concentration of leachates.

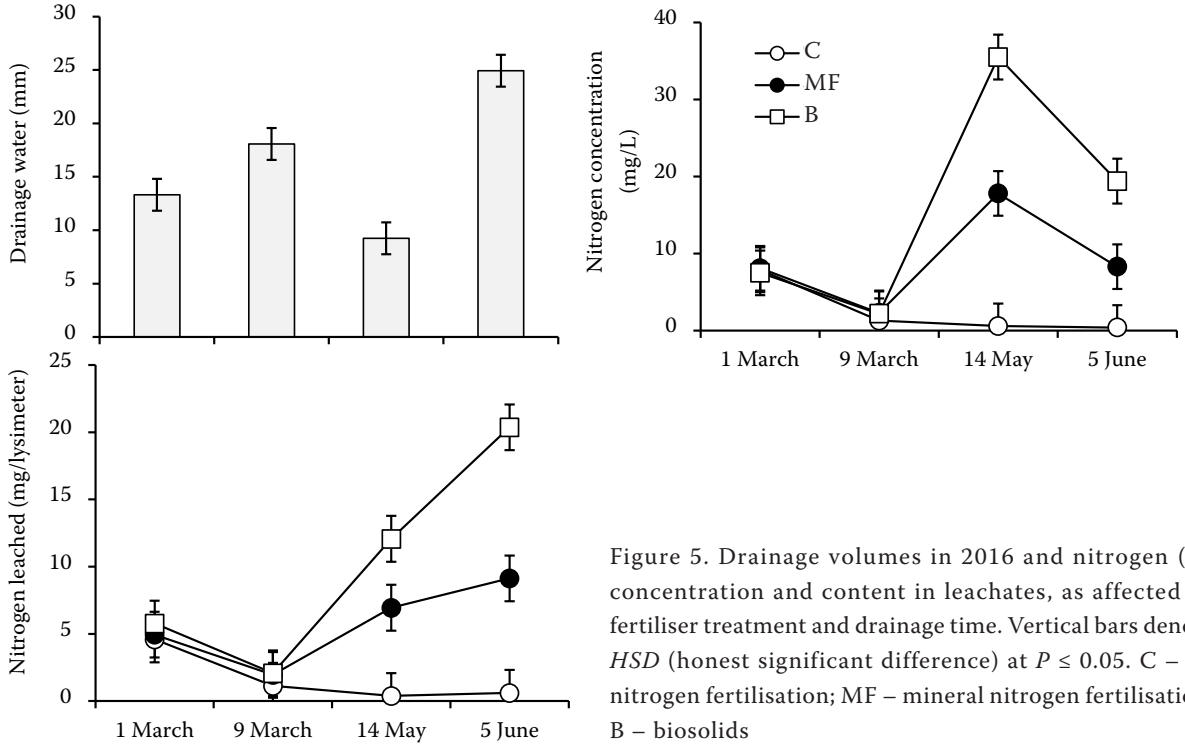

Figure 5. Drainage volumes in 2016 and nitrogen (N) concentration and content in leachates, as affected by fertiliser treatment and drainage time. Vertical bars denote *HSD* (honest significant difference) at $P \leq 0.05$. C – no nitrogen fertilisation; MF – mineral nitrogen fertilisation; B – biosolids

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Nitrogen losses reflected the NO_3^--N concentrations in drainage water and decreased from 1 to 9 March in all treatments (Figure 5). In the following leachates, N losses decreased progressively in controls, but increased markedly with both fertilisers. In all events the N loss was in the order C << MF < B, highlighting the fast and progressive N mineralisation of biosolids. Over the entire growth period, N losses with B were almost twice as high as with MF (40 vs. 23 mg/lysimeter), which corresponded to only 1.7% and 1.2% of the N taken up by plants, respectively. Low N leaching from biosolids and organic wastes-amended soils was also reported by Burgos et al. (2006) and Esperscheutz et al. (2016b). It can be therefore concluded that in Mediterranean regions, biosolids application to spring cereal crops can effectively replace mineral N, because the root net promptly accumulates the N progressively mineralised from the biosolids.

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