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Production of Biomass Crops Using Biowastes on Low-Fertility Soil: 2. Effect of Biowastes on Nitrogen Transformation Processes

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Abstract

Increasing production of biowastes, particularly biosolids (sewage sludge), requires sustainable management strategies for their disposal. Biosolids can contain high concentrations of nutrients; hence, land application can have positive effects on plant growth and soil fertility, especially when applied to degraded soils. However, high rates of biosolids application may result in excessive nitrogen (N) leaching, which can be mitigated by blending biosolids with other biowastes, such as sawdust. We aimed to determine the effects of biosolids and sawdust on growth and N uptake by sorghum, rapeseed, and ryegrass as well as N losses via leaching. Plants were grown in a greenhouse over a 5-mo period in a low-fertility soil amended with biosolids (1250 kg N ha^{-1}), biosolids-sawdust (0.5:1), or urea (200 kg N ha^{-1}). Urea application increased biomass production of sorghum and ryegrass but proved insufficient for rapeseed on low-fertility soil. Biosolids application increased plant N concentrations in ryegrass and rapeseed and increased N uptake into the seeds of sorghum, increasing seed quality. Biosolids application did result in lower N leaching compared with urea, irrespective of plant species, and N leaching was unaffected by mixing the biosolids with sawdust. There was an indication of biological nitrification inhibition in the rhizosphere of sorghum. Rapeseed had similar growth and N uptake into biomass in biosolids and biosolids-sawdust treatments and hence was the most promising species with regard to recycling fresh sawdust in combination with high rates of biosolids on low-fertility soil.

Core Ideas

- Mixing sawdust with biosolids did not reduce NO_3^- leaching irrespective of plant species.
- Compared with urea, biosolids application did not result in higher N loss via leaching.
- Results indicated biological nitrification inhibition of sorghum.

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WORLDWIDE, BIOWASTE PRODUCTION is increasing because of a rising population, agricultural intensification, and the need for improved food production (Río et al., 2011). Biowastes include crop residues, wood wastes, animal manures, food processing waste, and waste from municipal sewage treatment plants. Sustainable management strategies for disposal and recycling are required to reduce costs and negative environmental outcomes of landfill deposition (Amajirionwu et al., 2008). For some of these wastes, especially animal manure and biosolids (treated or stabilized sewage sludge), application to agricultural land is widely practiced and has shown positive effects on soil fertility and plant biomass (Miaomiao et al., 2009; Mok et al., 2013), along with improvements in soil chemical, physical, biological, and microbial properties (Cytryn et al., 2011; Rogers and Smith, 2007; Singh and Agrawal, 2008).

Biosolids are rich in organic matter and can contain essential plant nutrients such as nitrogen (N), phosphorus, sulfur, and potassium (Al-Dhumri et al., 2013). Therefore, land application of biosolids can have positive effects on soil fertility and plant growth (Corrêa, 2004; Petersen et al., 2003; Smith and Durham, 2002; Westerman and Bicudo, 2005), but there are drawbacks that need to be considered because biosolids may contain elevated concentrations of heavy metals, organic contaminants, and pathogens (Bolan et al., 2014). Because of the potential risks in using biosolids for agriculture, the notion of using biosolids to rebuild degraded land has become increasingly popular (Dere et al., 2012; Mbakwe et al., 2013; Meyer et al., 2001; Oladeji et al., 2013; Speir et al., 2003; Stehouwer et al., 2006). Biosolids application may promote topsoil development and enhance the reestablishment of vegetation, especially in degraded environments (Hearing et al., 2000; Lu et al., 2012).

Most N in biosolids is contained within the organic matter and thus is unavailable for plant uptake and not subject to leaching (Gilmour et al., 2003; Pu et al., 2012). Only small amounts of N are present in forms of nitrate (NO_3^-) and

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Abbreviations: BNI, biological nitrification inhibition; DW, dry weight.

ammonium (NH_4^+) (Eldridge et al., 2008); therefore, high rates of biosolids are necessary to establish plant growth and ecosystem function in low-fertility soils and degraded environments. However, high loads of potentially available N applied with biosolids can lead to excessive NO_3^- leaching, which can negatively affect waterways (Smith, 2003). Therefore, best management practices have to be followed to ensure protection of soil, food, feed, and waterways. Total and mineral N concentrations in biosolids vary with type and treatment of biosolids, which has been extensively reviewed recently by Rigby et al. (2016). Several factors have been identified influencing N transformations in biosolids-amended soil, such as biosolids type, C/N ratio, application rate, soil texture and organic matter content, soil temperature, soil moisture, and soil pH (Rigby et al., 2016).

The negative effects of biosolids addition to soil can be mitigated by mixing biosolids with other biowastes. Positive results have been reported by adding biochar and wood wastes (Ammari et al., 2012; Knowles et al., 2011). Biosolids-sawdust mixtures have a beneficial effect on plant growth and soil aggregate stability while reducing NO_3^- leaching (Bugbee, 1999; Sandoval et al., 2012). In combination with other organic wastes, sawdust has the potential to improve the physical, chemical, and nutritional properties of soils (Paramashivam et al., 2016; Sandoval et al., 2012). Therefore, blending biowastes can enable recycling strategies that result in decreased landfill deposits. The optimal ratios of mixing other biowastes with biosolids have to be determined to avoid negative effects on plant growth (Schmidt et al., 2001).

Rapid nitrification, the conversion of NH_4^+ to NO_3^-, can result in the inefficient use of N due to NO_3^- loss from agricultural systems through leaching or the emission of N_2O after subsequent denitrification (Robertson and Groffman, 2015). By restricting nitrification, N retention in the soil is increased because NH_4^+ is less likely to be lost via leaching and denitrification (Subbarao et al., 2013). Several plant species have shown the potential to inhibit nitrification, which could further mitigate N loss from agricultural systems (Fillery, 2007; Subbarao et al., 2009, 2013). Biological nitrification inhibition (BNI) through exudation of nitrification inhibiting compounds from roots has been shown for tropical pasture plants (Subbarao et al., 2009), but biomass crop species such as sorghum (*Sorghum bicolor* L.) and rapeseed (*Brassica napus* L.) have also shown potential to inhibit nitrification (Brown and Morra, 2009; Zakir et al., 2008). Growing crops with BNI properties on biosolids-amended soils could therefore influence N transformation processes and reduce the risk of N leaching after high rates of biosolids application.

We aimed to determine the effects of biosolids and sawdust on the growth and N uptake by sorghum, rapeseed, and ryegrass as well as N losses via leaching. Following high biosolids application rates on low fertility soil, we hypothesize that potential loss of N can be mitigated by blending biosolids with sawdust and by the selection of plant species with high N requirements or nitrification inhibition properties. The focus of this manuscript is on plant and soil N transformation processes; nutrients and trace elements are discussed in Part 1 of this study (Esperschütz et al., 2016a)

Materials and Methods

Experimental Setup

An experiment was set up at the Lincoln University plant growth facility, as described in detail by Esperschütz et al. (2016a). In brief, low-fertility soil, as defined according to its low Olsen P of 11 mg L^{-1}, was collected from a marginal farm area (40°45′56″ S, 175°54′42″ E) and placed into small lysimeters (25 cm in diameter; of 29 cm). To measure NO_3^- leaching, a leachate-sampling device was installed in the bottom of each lysimeter. Lysimeters were incubated at ambient conditions in a greenhouse for 14 wk before treatment application. Rapeseed (*Brassica napus* L. 'MAKRO'), sorghum (*Sorghum bicolor* L. Moench 'Sudanese'), and ryegrass (*Lolium multiflorum* Lam. Feast II tetraploid Italian ryegrass, 2 g) were grown in four different treatments (control, biosolids, biosolids-sawdust, urea) in individual lysimeters, randomized within the experiment with four replicates for rapeseed and sorghum and six replicates for ryegrass, respectively.

Biosolids were collected from settlement ponds of the Kaikoura Sewage Treatment Plant; sawdust was obtained from an adjacent wood-waste disposal area (Kaikoura, New Zealand, 42°21′37.40″ S, 173°41′27.35″ E). Biosolids (untreated pond sludge, characterized as Grade "Bb" according to NZWWA [2003]) were homogenized thoroughly after sieving ($\leq$10 mm). Fresh *Pinus radiata* sawdust was used to mix with the biosolids. A characterization of soil, sawdust, and biosolids is presented in Esperschütz et al. (2016a). Fresh biosolids (245 g dry weight [DW]) and biosolids mixed with sawdust (245 g DW + 123 g DW) were applied at rates of 1250 kg N ha^{-1}, respectively, with biosolids application equivalent to 50 t ha^{-1} DW. Urea was applied four times over the experimental period (50 kg N ha^{-1} equivalent) up to a total amount of 200 kg N ha^{-1}. Seeds were sown directly into the lysimeters after urea and biosolids application.

The experiment was maintained for 18 wk. The temperature in the greenhouse ranged between 9 and 20°C during nighttime (10 PM until 6 AM) and between 14 and 28°C during the daytime. Using automatic and manual irrigation, soil was maintained at near-field capacity conditions. A total of 2160 mm of irrigation was applied to rapeseed, 1190 mm to sorghum, and 1060 mm to ryegrass.

Analyses and Measurements

The amount of leachate was sampled and recorded weekly throughout the experimental period; aliquots were stored at −20°C until further analyses. Nitrate-N (NO_3^-–N), nitrite-N (NO_2^-–N), and ammonium-N (NH_4^+–N) were determined using a flow injection analyzer (FIA FS3000 twin channel analyzer, Alpkem). Evapotranspiration was calculated as the volume of water irrigated (mL), reduced by the volume recovered as drainage (mL), and subsequently added week by week over the experimental period.

The biomass of ryegrass was repeatedly harvested and analyzed for its macro- and micronutrient speciation throughout the experiment (Esperschütz et al., 2016b). In this study, the cumulative ryegrass biomass was calculated based on eight harvests performed fortnightly with the first harvest 4 wk after sowing. The cumulative ryegrass biomass was compared with the biomass of sorghum and rapeseed, respectively, obtained from a final destructive harvest of all lysimeters after 18 wk. The total plant biomass of sorghum and rapeseed was weighed and oven-dried at 70°C until a constant weight was achieved. Dried plant parts were further separated into roots, leaves, and seeds and ground to a fine powder using a Retch ZM200 grinder for analyses. Soil that has been attached to the plant roots ≤2 mm was considered as rhizosphere soil. Rhizosphere soil was sieved (≤5 mm) before chemical analyses. Based on the different harvesting protocol for ryegrass, no seeds or roots were harvested from *L. multiflorum* plants. Total C and N in plant and soil material were analyzed from ground material using a CNS-2000 Element Analyzer (LECO Australia Pty Ltd).

The soil inorganic N speciation was determined using a KCl extraction from fresh soil (4°C) within 4 d after harvest according to (Blakemore et al., 1987). After adding 40 mL of a 2 mol L^{-1} KCl reagent to 4 g of soil, the solution was shaken on an end-over-end shaker for 1 h, centrifuged at 827 *g* for 10 min, and filtered through Whatman 41 filter paper. Nitrate-N (NO_3–N) and ammonium-N (NH_4–N) were determined using a flow injection analyzer (FIA FS3000 twin channel analyzer, Alpkem).

Statistical analyses were based on four individual replicates for rapeseed and sorghum and six replicates for ryegrass, respectively. Using SPSS 22 (IBM SPSS statistics), ANOVAs were performed followed by Duncan's post hoc tests to identify homogenous subsets for $\alpha = 0.05$. Significance between treatments during the 18-wk period were investigated using a full factorial, multivariate model followed by Duncan's post hoc tests. Results were illustrated in SigmaPlot 11.0 (Systat Software Inc.).

Results

High rates of biosolids application to low-fertility soil resulted in a significant growth response of rapeseed, sorghum, and ryegrass compared with control treatments (Fig. 1a). Rapeseed produced negligible biomass in the control and urea treatments due to a lack of nutrients available to maintain the growth of this species (Esperschütz et al., 2016a). Mixing sawdust with biosolids reduced the growth of sorghum and ryegrass compared with biosolids-only, whereas similar biomass was harvested in rapeseed treatments. Urea application increased biomass production of sorghum and ryegrass but had no effect on rapeseed grown on low-fertility soil.

Biosolids application significantly increased total N uptake into plant biomass in combination with rapeseed and ryegrass (Fig. 1b). Higher N contents in sorghum and ryegrass were seen in urea treatments compared with control, biosolids-sawdust, and biosolids treatments. For rapeseed and sorghum, the N uptake into total plant biomass was similar in biosolids-sawdust and biosolids treatments, whereas ryegrass grown with the biosolids-sawdust mix showed lower N uptake compared with biosolids.

Due to the different harvesting procedure for ryegrass, no seeds or roots were harvested from *L. multiflorum* plants at the end of the experiment. The scarce growth of rapeseed in control and urea treatments has not resulted in seed production in these treatments; hence, seeds could only be harvested from rapeseed biosolids-sawdust and biosolids treatments. No difference was detected in the seed N concentration between biosolids and biosolids-sawdust treatments in rapeseed (2.9 and 3.0%, respectively). The percentage distribution of N between sorghum leaves, seeds, and roots is shown in Fig. 2. Urea application increased leaf-N by up to 74.8% compared with the control, whereas a decrease was observed in seed-N (−4.6%) and root-N (−20.6%) concentration. Biosolids-sawdust application caused an increase in seed-N up to 15.7% relative to controls. Nitrogen was further partitioned into the seeds in the biosolids alone treatment (28.9%).

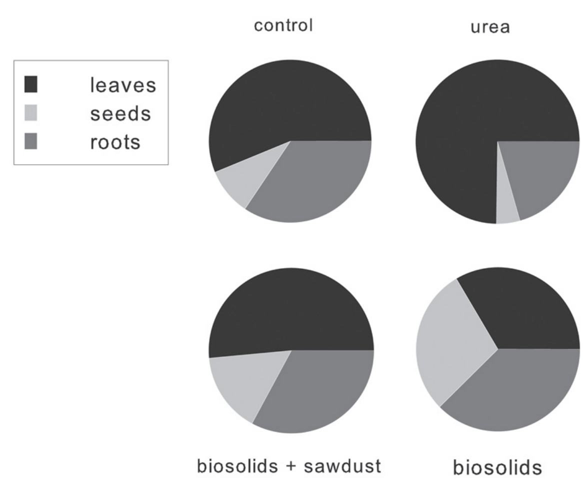

Fig. 2. Distribution of N (%) between sorghum (*S. bicolor*) leaves, seeds, and roots at final harvest after 18 wk.

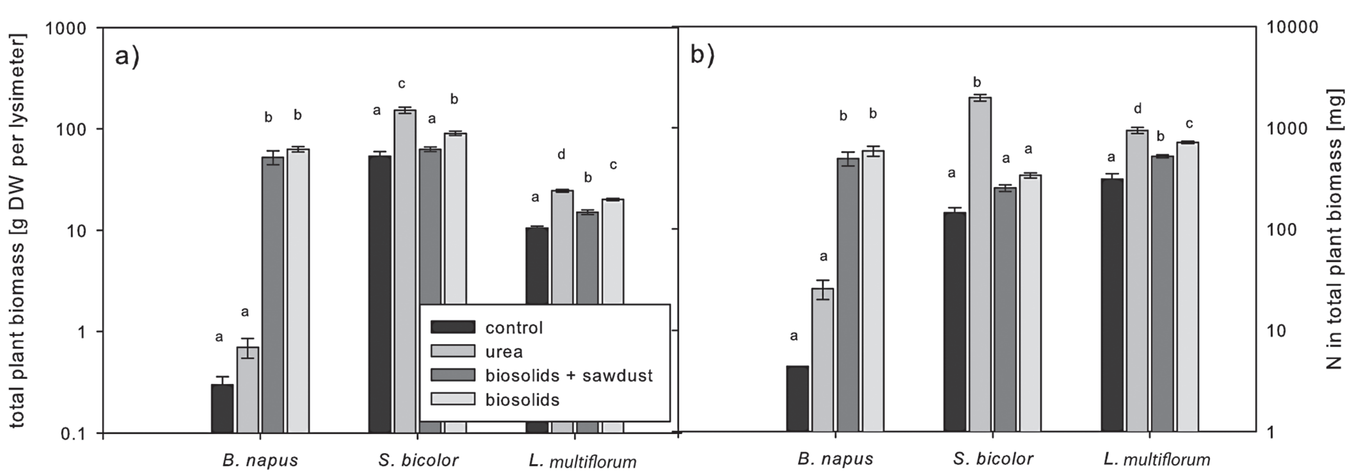

Fig. 1. Total plant biomass (including seed and root biomass) at the end of the experiment (a) and total N recovered from plant biomass (b) from sorghum (*S. bicolor*), rapeseed (*B. napus*), and ryegrass (*L. multiflorum*). Different levels of significance $p \leq 0.05$ are represented by lowercase letters.

At the end of the 18-wk experiment, NH_4^+ concentrations were below detection limit (0.1 mg L^{-1}, equivalent to 5 mg kg^{-1}) in the rhizosphere of all plant species. Nitrate was not significantly different between treatments in rapeseed and ryegrass (12.7–20.5 mg kg^{-1}), whereas NO_3^- concentrations were below detection limit (0.1 mg L^{-1}, equivalent to 5 mg kg^{-1}) in the rhizosphere of sorghum (data not shown).

Drainage and evapotranspiration of different plant species in combination with soil treatments were calculated for each week. Under ryegrass, no significant difference was detected between treatments up to Week 4. Five weeks after the start of the experiment, the cumulative drainage was lowest in biosolids treatments (79 mm) compared with biosolids-sawdust (105 mm) until the end of the experiment (366 and 476 mm, respectively) (Fig. 3a).

In combination with rapeseed, the cumulative drainage at the end of the experiment was significantly higher in urea and control treatments (670 and 676 mm, respectively) compared with biosolids and sawdust-biosolids treatments (450 and 477 mm, respectively) (Fig. 3b), with the differences between these two groups consistent after 8 wk ($p \leq 0.05$).

Drainage from the sorghum treatments ranged from 289 mm in urea treatments to 392 mm in the biosolids-sawdust treatments at the end of the experiment (Fig. 3c). The urea and biosolids treatments had similar drainage compared with the control until Week 15 but separated significantly during the last 3 wk, with negligible drainage recovered from urea treatments. In biosolids-sawdust treatments, drainage was consistently higher compared with other treatments after 5 wk.

At the end of the experiment, the cumulative evapotranspiration of ryegrass was significantly higher in biosolids treatments (627 mm) compared with the control and biosolids-sawdust treatments (532 and 516 mm, respectively). The cumulative evapotranspiration of urea and biosolids was consistently higher after the first 8 wk (302 and 312 mm, respectively) compared with biosolids-sawdust (262 mm) (Fig. 4a), whereas control treatments showed similar evapotranspiration as biosolids-sawdust (276 mm). In the rapeseed treatments, a higher cumulative evapotranspiration was detected in biosolids and biosolids-sawdust treatments, consistently significant after 10 wk (Fig. 4b). Sorghum had a consistently lower evapotranspiration in the biosolids-sawdust treatments compared with all other treatments after Week 6 (585 mm). The urea, control, and biosolids treatments used similar amounts of water during the experiment, with the urea treatments significantly higher (676 mm) at Week 18 compared with the control (627 mm) and biosolids (638 mm) treatments (Fig. 4c). Irrespective of soil treatments, cumulative evapotranspiration was highest in rapeseed (814–1017 mm) compared with sorghum (585–675 mm) and ryegrass (516–627 mm).

Leaching of NO_3^- varied depending on plant species, whereas NO_2^- and NH_4^+ concentrations were always below detection limits (data not shown). No differences were observed between biosolids-sawdust and biosolids treatments for any plant species in the experiment. Rates of NO_3^- detection in leachate were 135 to 148 mg NO_3^- under rapeseed (Fig. 5b), 218 to 220 mg NO_3^- under sorghum (Fig. 5c), and 79 to 115 mg NO_3^- under ryegrass (Fig. 5a).

No differences were detected in the amount of NO_3^- leached from soil treatments with ryegrass during the first 3 wk of the experiment (Fig. 5a), but significantly higher contents of NO_3^- were collected from drainage of biosolids-sawdust and urea treatments compared with the control from Week 5 onward. Leaching of NO_3^- from biosolids treatments was higher compared with the control but lower compared with urea and biosolids-sawdust until the end of the experiment. However, no significant differences were recorded between either of these treatments. After 18 wk, the total NO_3^- leached was 116 and 113 mg from urea and biosolids-sawdust treatments, respectively, whereas 95 mg was leached from biosolids treatments and 79 mg leached from the control.

In combination with rapeseed, the highest amount of cumulative NO_3^- (796 mg) leached in drainage was detected in the urea treatments, followed by control treatments (357 mg) and then biosolids and biosolids-sawdust treatments (162 and 180

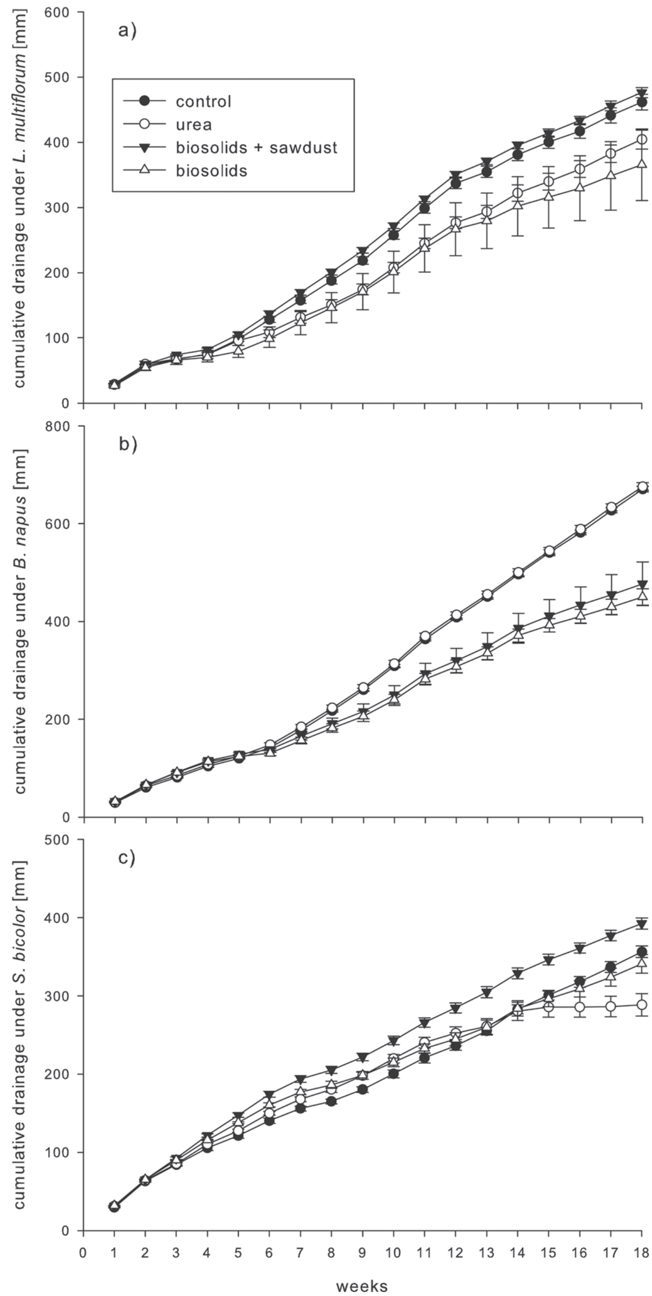

Fig. 3. Cumulative drainage [mm] of ryegrass (*L. multiflorum*) (a), rapeseed (*B. napus*) (b), and sorghum (*S. bicolor*) (c) during the experimental period. Differences $p \leq 0.05$ are represented by nonoverlapping SEM.

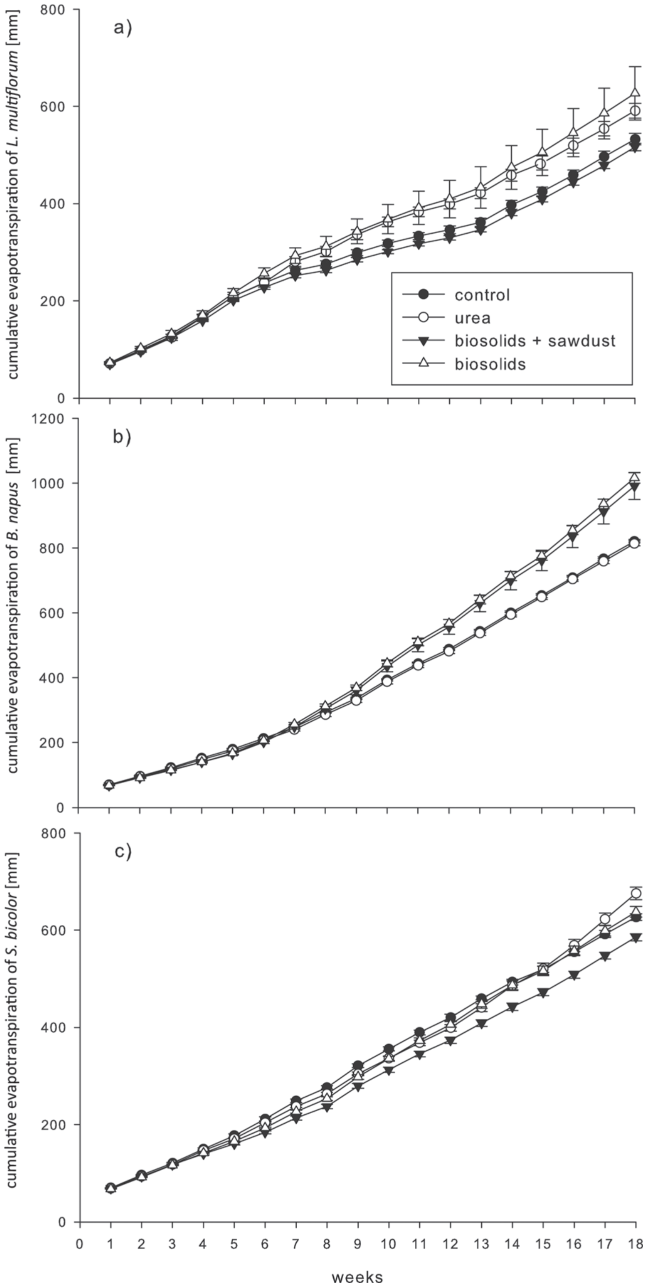

Fig. 4. Cumulative evapotranspiration [mm] of ryegrass (*L. multiflorum*) (a), rapeseed (*B. napus*) (b), and sorghum (*S. bicolor*) (c) during the experimental period. Differences ($p \leq 0.05$) are represented by nonoverlapping SEM.

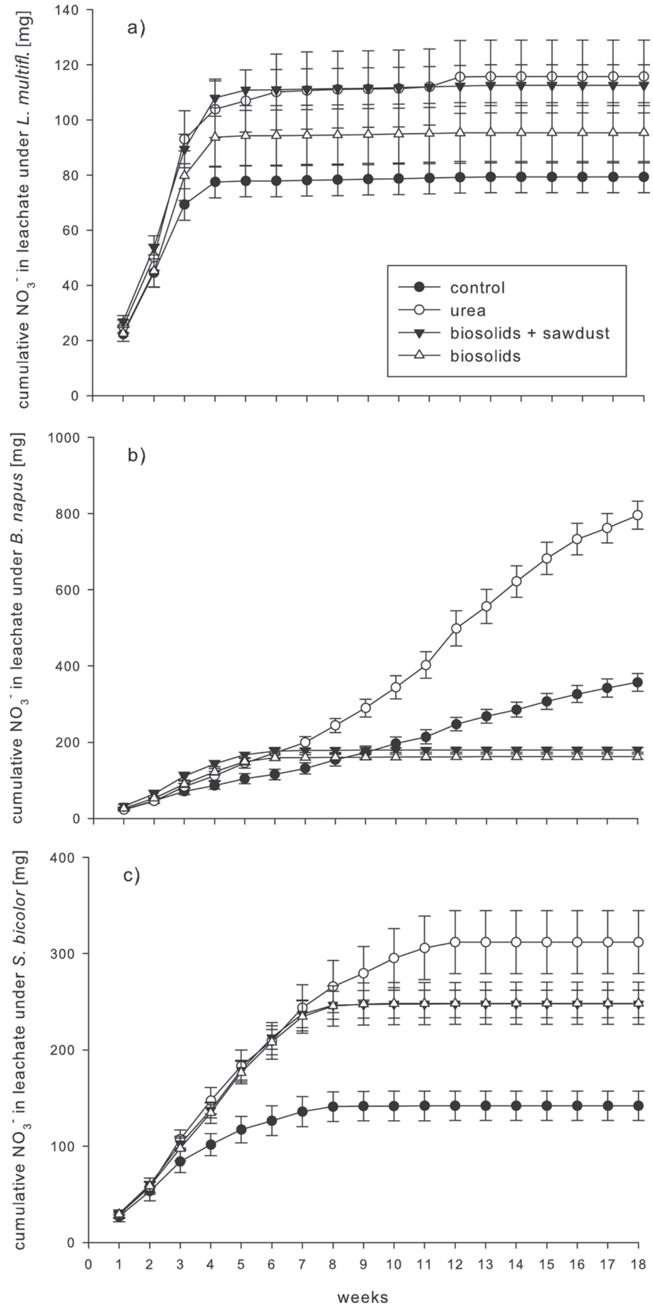

Fig. 5. Cumulative NO_3^- in leachate [mg] under ryegrass (*L. multiflorum*) (a), rapeseed (*B. napus*) (b), and sorghum (*S. bicolor*) (c) vegetation during the experimental period. Differences ($p \leq 0.05$) are represented by nonoverlapping SEM.

mg, respectively) (Fig. 5b). Urea treatments leached significantly higher amounts than the other treatments after only 8 wk, whereas control treatments were consistently higher compared with biosolids and biosolids-sawdust after 12 wk. No differences were found between biosolids and biosolids-sawdust treatments throughout the 18-wk experimental period.

Cumulative NO_3^- leached from sorghum controls (142 mg) was lower than the urea, biosolids, and biosolids-sawdust treatments (Fig. 5c). Higher amounts of NO_3^- were recovered from urea treatments (312 mg) but were not significant ($p = 0.076$) compared with biosolids and biosolids-sawdust treatments.

Discussion

Biosolids and Sawdust Application Increase Growth and Biomass N in Rapeseed

Rapeseed showed a very scarce biomass production in control and urea treatments, which was likely the result of other limiting nutrients than N, mainly S and P (Esperschütz et al., 2016a). Growth of rapeseed increased due to biosolids-sawdust and biosolids. A biomass increase of rapeseed after biosolids application confirms findings from Shaheen and Tsadilas (2013), who reported a biomass increase after different rates of biosolids were applied in a pot experiment using low-fertility soil comparable (to some extent) to the soil used in our study.

Nitrogen accumulation in the total plant biomass reflected results from total plant biomass. Biosolids and biosolids-sawdust application increased the total plant N content and thereby enhanced plant quality. At the end of the growing period, the total N content in rapeseed biomass was equivalent to 110 kg ha^{-1} in the biosolids and biosolids-sawdust treatments. In rapeseed, neither plant growth, plant N content, nor N uptake into seeds was influenced in biosolids-sawdust treatments compared with pure biosolids application. This may indicate that mixing sawdust with biosolids is a suitable way of recycling fresh sawdust in combination with high rates of biosolids on a low-fertility soil in combination with rapeseed.

The total mass of NO_3^- leached in rapeseed was higher in the control and urea treatments compared with biosolids and biosolids-sawdust treatments. A lack of plant establishment in urea and control treatments (<1 g) resulted in NO_3^- leaching comparable to bare soil. Compared with pure biosolids treatments, biosolids-sawdust application did not affect plant growth, drainage, or evapotranspiration during the experimental period, as shown for ryegrass and sorghum. This may indicate that rapeseed is more sensitive to other macronutrients, such as P and S, as discussed elsewhere (Esperschütz et al., 2016a), and that N was not the limiting factor for rapeseed.

Decreased Growth and N Uptake of Ryegrass in Sawdust Treatments

Biosolids as well as urea application resulted in a total biomass production of ryegrass similar to the reported average for a Feast II Italian ryegrass cultivar (Hanson et al., 2006), as discussed in detail by Esperschütz et al. (2016b). Biosolids-sawdust application in combination with ryegrass caused a decrease in plant growth and reduced N accumulation into plants. As discussed in Esperschütz et al. (2016b), our results showed that biosolids and biosolids-sawdust resulted in less growth compared with urea application. Only a limited amount of the total N applied with biosolids (1250 kg ha^{-1}) was immediately plant available, with most of the N in biosolids locked up in organic compounds. Such organic N needs to undergo (microbial) transformation processes to become available (Sommers, 1977). In addition, other properties of the biosolids, such as elevated trace elements or salinity, may have reduced the effectiveness of the added N.

Throughout the 18-wk experimental period, available N from urea and biosolids application was taken up for biomass growth, resulting in a better growth response, lower drainage, and higher evapotranspiration compared with control treatments (Di Paolo and Rinaldi, 2008; Kim et al., 2008). Higher drainage in biosolids-sawdust treatments compared with biosolids or urea application is compatible with reduced plant biomass (due to less available N) and therefore less need of water to support biomass growth. Leaching of NO_3^- occurred mainly in the first weeks of the experiment, immediately after treatment application. This was probably due to leaching through rather bare soil until significant plant biomass was established after 4 wk. After biomass harvests began, drainage was sampled weekly with NO_3^- <0.5 mg, which indicates the utilization of available N by ryegrass plants in all treatments. Biosolids application at a rate of 1250 kg ha^{-1} N hence did not result in higher N loss via leaching compared with 200 kg N ha^{-1} applied with urea.

Using sawdust in combination with biosolids provided no additional benefit over biosolids alone with regard to plant growth and evapotranspiration. This could have been related to an immobilization of N by heterotrophic bacteria that were likely stimulated by the C-rich sawdust (Robertson and Groffman, 2015; Schmidt et al., 2001) or to adsorption reactions of NO_3^- with functional groups on sawdust, as suggested by Harmayani and Anwar (2012). Because there is insufficient N in sawdust to allow microbes to build proteins, they must accumulate N from their environment, in this case from biosolids; hence, biosolids-N might have already been immobilized by sawdust when sawdust was mixed with biosolids before application.

Mixing biosolids with sawdust caused a reduction of plant-available N, which resulted in reduced plant growth compared with biosolids and urea treatments; hence, lower evapotranspiration was calculated on the basis of total plant biomass. This is in contrast to a study growing a hybrid ryegrass (*Lolium* × *hybridum* Hausskn. 'Belinda') in combination with different biosolids and sawdust ratios applications in a degraded soil (Sandoval et al., 2012). Whereas biosolids-sawdust application in our study (using Feast II Italian ryegrass) showed a negative growth response, comparable treatments in Sandoval et al. (2012) indicated a biomass increase. We therefore suggest the influence of soil type and fertility, as well as the *Lolium* species and cultivar type, need to be taken into account when determining biosolids-sawdust ratios to optimize plant growth and soil regeneration.

Response of Sorghum to Mineral and Organic Soil Amendments

Both urea and biosolids enhanced the biomass production of sorghum by providing N sources readily available for plant growth (Esperschütz et al., 2016a). However, higher growth response was obtained in urea treatments, likely due to higher amounts of N readily available for plant uptake compared with biosolids, as discussed for ryegrass above. The high biomass response of sorghum to mineral N (urea) fertilization was in accordance with Fellet and Marchiol (2011), who suggested that higher biomass due to mineral fertilization was caused by a longer vegetative period, delaying the senescence of the canopy. In our study, however, a mineral fertilization rate of 200 kg N ha^{-1} resulted in a growth response similar to control treatments without N addition, carried out by Turgut et al. (2005). This may indicate that in addition to N limitation, the total plant yield was likely affected by the low fertility soil used in our study, specifically the lower availability of macronutrients P and K.

In our study, sorghum had a high response to N (urea) fertilization, with high N uptake into aboveground vegetative plant parts. This result can be of potential interest at sites with high N because plants showed a fast uptake of available N, hence removing N susceptible to leaching. Whereas biosolids and biosolids-sawdust application did not cause a significant N increase in the total sorghum plant, a clear shift could be observed in the plant N distribution toward higher contents of N in seeds, indicating an increase of seed quality and a change in the plant N translocation through organic amendment treatments (Fig. 2).

In sorghum, more drainage in biosolids-sawdust treatments was observed compared with biosolids or urea treatments. This is in accordance with lower evapotranspiration and is likely related to less available N and therefore less need of water for biomass growth. Biosolids application increased evapotranspiration (less drainage) compared with the control treatment, which is consistent with the findings of (Fiasconaro et al., 2013), who showed an increased evapotranspiration of leguminous plants after sewage sludge treatment. Using sawdust in combination with biosolids had no benefit with regard to plant growth and evapotranspiration in sorghum. Directly after biosolids application, an increasing amount of NO_3^- (up to 33 mg) in leachate in the first 4 wk could be explained by the phase of plant development. The amounts of NO_3^- recovered in leachate was likely the result of N flow through the bulk soil before significant plant biomass was established.

Neither NH_4^+ nor NO_3^- was measured in the rhizosphere of sorghum after 18 wk, whereas in both rapeseed and ryegrass, rhizosphere NO_3^- was detected between 15 and 20 mg (data not shown). However, no difference was found in rhizosphere NO_3^- at the end of the experiment in rapeseed and ryegrass treatments, which may indicate a contamination of rhizosphere with bulk soil, making NO_3^- less accessible for roots. Because large amounts of NO_3^- might have been leached from sorghum bulk soil before plant growth and preferential flow, less "bulk soil NO_3^-" was available at the end of the experiment to "contaminate" the rhizosphere sample.

Commonly, NO_3^- and NH_4^+ are the main sources of N for plant growth (Robertson and Groffman, 2015), with uptake mechanisms varying between plant species and growth stages and depending on environmental parameters, along with plant physiological processes (Ruffel et al., 2014). Nutrient availability in the rhizosphere may be influenced by evapotranspiration or differences in the preferential flow due to different root system architecture (Allaire et al., 2011; Mitchell et al., 1995). In our study, sorghum plants may have compensated high N requirements by mobilizing high amounts of soil-available N in addition to utilizing biosolids-available N. In this context, root exudates may have been involved, influencing root–microbe interactions and thereby inhibiting nitrification (Nardi et al., 2000; Subbarao et al., 2013). This would be in accordance with recent findings, where nitrification-inhibiting compounds have been detected in root exudates of sorghum (Zakir et al., 2008). Biological nitrification inhibition (BNI) would have decreased the NO_3^- in rhizosphere with NH_4^+ serving as major N source taken up by the plants. In a nitrification assay, performed from rapeseed, ryegrass, and sorghum control soil, sorghum showed lower NO_3^- and higher NH_4^+ concentrations compared with rapeseed and ryegrass (Supplemental Fig. S1). This may indicate the presence of BNI compounds due to root exudation and hence an inhibition of nitrifying bacteria, oxidizing NH_4^+ to NO_3^-. In this context, methyl 3-(4-hydroxyphenyl) was isolated as an active BNI compound in sorghum root exudates and was found in higher concentrations in the presence of NH_4^+ (Zakir et al., 2008). However, this could not be verified in our study because a nitrification assay was performed only from control soil, but this finding opens new lines of research studying urea and biosolids application in combination with sorghum plants.

Effect of Adding Sawdust to Biosolids on N Leaching

No significant difference between biosolids and biosolids-sawdust treatments were detected regarding the amount of NO_3^- leaching in combination with any plant species. Nitrate was recovered in leachate from both treatments, which is in contrast to other studies (and discussed above with regard to NO_3^- immobilization by sawdust) because this process should result in less NO_3^- leached in biosolids-sawdust treatments. An inhibitory effect of sawdust regarding N and other elements was shown by Kováčik et al. (2013) when fresh sawdust had been applied with dry pig manure to reduce nutrient mobility in soil. However, we suggest that in our study the available inorganic N applied with biosolids has been immobilized, whereas the NO_3^- recovered in leachate was likely to be of soil origin, because throughout the experiment NO_3^- leaching could be observed from more or less bare soil (i.e., rapeseed control treatments).

The total N in soil at the beginning of the experiment was calculated as 7200 kg ha^{-1}, which was increased by 1250 kg ha^{-1} in biosolids and biosolids-sawdust treatments, and 200 kg ha^{-1} in urea treatments, respectively. Although, depending on the plant species, between 20 and 150 kg ha^{-1} of N was removed with plant biomass and between 10 and 80 kg ha^{-1} was lost via leaching, any change in soil N at the end of the experiment will be in the order of 3%. Although we measured the residual N concentration in the rhizosphere soil, we could not resolve this difference, particularly in the case of the biosolids-amended soils where there would have been significant redistribution of N within the soil profile.

Conclusions

Biosolids application to low-fertility soil provided sufficient nutrients to ensure adequate growth of all plant species in this experiment. Biosolids application rates equivalent to 1250 kg N ha^{-1} did not result in an increased N loss via leaching compared with urea treatments. The use of sawdust did not reduce NO_3^- leaching but instead may have immobilized and reduced available N for plant growth in combination with sorghum and ryegrass. Further investigation of different biosolids/sawdust ratios and different plant species could increase the prevalence of sawdust and biosolids recycling on land, instead of increasing landfill deposits. The BNI properties of sorghum affected rhizosphere N but showed no effect on NO_3^- leaching. In this context, mixing biosolids with soil instead of topsoil applications could be of interest for future experiments. To investigate N leaching and BNI, stable isotope experiments could identify different sources of NO_3^- and further identify the effect of plant exudates on N transformation processes in combination with biowastes. Future work should involve field measurements in large plantations, where edge effects are less important than in a lysimeter experiment.

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