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NITROGEN WASHING FROM C3 AND C4 COVER GRASSES RESIDUES BY RAIN

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SUMMARY

Crop species with the C₄ photosynthetic pathway are more efficient in assimilating N than C₃ plants, which results in different N amounts prone to be washed from its straw by rain water. Such differences may affect N recycling in agricultural systems where these species are grown as cover crops. In this experiment, phytomass production and N leaching from the straw of grasses with different photosynthetic pathways were studied in response to N application. Pearl millet (Pennisetum glaucum) and congo grass (Brachiaria ruziziensis) with the C₄ photosynthetic pathway, and black oat (Avena Strigosa) and triticale (X Triticosecale), with the C₃ photosynthetic pathway, were grown for 47 days. After determining dry matter yields and N and C contents, a 30 mm rainfall was simulated over 8 t ha⁻¹ of dry matter of each plant residue and the leached amounts of ammonium and nitrate were determined. C₄ grasses responded to higher fertilizer rates, whereas N contents in plant tissue were lower. The amount of N leached from C₄ grass residues was lower, probably because the C/N ratio is higher and N is more tightly bound to organic compounds. When planning a crop rotation system it is important to take into account the difference in N release of different plant residues which may affect N nutrition of the subsequent crop.

Index terms: Cover crops, nitrogen fertilization, nutrient cycling.

RESUMO: LAVAGEM DO NITROGÊNIO DE RESÍDUOS DE PLANTAS C₃ E C₄ PELO CHUVA

Espécies com via fotosintética C₄ são mais eficientes na assimilação de N que plantas C₃, o que resulta em diferentes resistências do N do tecido à possibilidade de lavagem por...
INTRODUCTION

One of the most important aspects to consider when choosing cover crop species is the N accumulation capacity, either by atmospheric N₂ fixation or uptake from soil, or both. However, N mineralization dynamics in cropping systems depend on factors such as the nature of organic N, C/N ratio of plant residues, N amount in the system and degree of contact of the tissues with soil colloids, as well as temperature, aeration, and moisture (Rosolem et al., 2004). The use of cover crops may result in significant increases not only in surface mulch and soil organic matter contents but also in nutrient cycling (Rosolem et al., 2006). Cultivated species of the Poaceae family (grasses) usually respond well to applied N. However, for grasses grown as cover crops the capacity to accumulate N is as essential as the speed at which N contained in plant residues is washed back to the soil with rains or mineralized after desiccation, which will determine when this N will be available for the following crop.

Some grasses have the C₃ photosynthetic pathway for C fixation, e.g., black oat (Avena strigosa), while others show the C₄ photosynthetic pathway, such as pearl millet (Pennisetum glaucum). This physiological discrimination influences N-release dynamics from the phytomass after the plants are cut (Rosolem et al., 2005). Tropical grasses e.g., as pearl millet, sorghum and Congo grass, have the C₃ photosynthetic pathway and are more efficient in phytomass accumulation per unit of N taken up, compared with C₄ plants such as wheat (Triticum aestivum) and black oat (Avena strigosa) (Taiz & Zeiger, 2002). Hence, after desiccation, the N-release dynamics from the phytomass of cover grasses may differ between C₃ and C₄ species. As a result, N supply to the subsequent crop may vary in each crop rotation system, which should be taken into account in the plant residue management. The use of cover crops may result in significant increases not only in surface mulch and soil organic matter contents but also in nutrient cycling (Cantarella et al., 1997). One of the desirable traits in selecting cover crops species is the amount of N accumulated in the phytomass (Oliveira et al., 2002). Nevertheless, the proportion of N that will actually be used by the subsequent crop will depend on the synchronization between N release from the straw and the demand of the succeeding crop.

According to Taiz & Zeiger (2002), in C₄ photosynthetic pathway plants, NO₃⁻ is reduced and assimilated in leaf mesophyll cells, and CO₂ is incorporated in vascular bundle sheath cells, which increases the physiological efficiency in this plant group compared to C₃ plants, considering the use of light energy to assimilate N. Moreover, less than 10 % of the total N in C₄ plants leaves is used in the formation of the RuBP Carboxylase Oxygenase enzyme (Rubisco), and between 2 and 5 % is used in the formation of the PEP-carboxylase enzyme, whereas in C₃ plants Rubisco immobilizes from 20 to 30 % of the total N in the leaf tissue. The Rubisco enzyme accounts for approximately 50 % of the soluble proteins in the leaves of C₃ plants, compared with less than 10 % in C₄ plants. Therefore, there is greater N availability for the growth and development of plant tissues in C₄ plants, whereas in C₃ plants Rubisco works as a reserve of soluble N (Pimentel, 1998). Therefore, under equivalent environmental conditions, the shoots of C₃ plants theoretically contain more available NH₄⁺ per dry matter mass unit than C₄ plants (foloni et al., 2009). Since N can be found in distinct forms in the leaf tissue of C₃ and C₄ plants, rain leaching of this nutrient from plant residues may be different in these groups of plants.

One hypothesis to explain the greater N-NH₄⁺ leaching from plant residues in triticale and black oat (grasses) and in sunn hemp (legume) is the nature of N bounding to organic compounds resulting from
the C₃ photosynthetic pathway for C assimilation (Rosolem et al., 2005). The intensity of N leaching from the straw of these species was higher than from C₄ species under weak rainfall and tended to be similar to that of C₃ plants, such as millet, guinea sorghum and congo grass under more intense rainfall. The amounts of N in C₃ were higher than in C₄ leaf tissue before rainfall. Thus, the higher the N content in the straw, the higher the amount of N washed away with rainwater, by a 70 mm rainfall (Rosolem et al., 2005).

If N assimilation and accumulation are different in C₃ and C₄ plants resulting in different N concentrations in the tissue and different dynamics in N release under rainfall, N cycling potential may be different for these species when grown as cover crops.

The objective of the experiment was to evaluate biomass production, N accumulation and N washed from residues of two C₃ species (millet and congo grass) and two C₃ species (black oat and triticale) by simulated rain, as related to N fertilization.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse in Botucatu, State of São Paulo, Brazil. The soil was collected from the arable layer (0–20 cm) of a dystroferric Red Latosol (Embrapa, 1999) with 63 % sand, 4 % silt, and 33 % clay. The soil was air-dried and passed through a 2 mm sieve. Chemical analyses (Raij et al., 2001) showed pH (CaCl₂) 4.1, 17.0 g dm⁻³ O.M., 3.0 mg dm⁻³ P (resin), 200 mg dm⁻³ K, 69.0 mmolc dm⁻³ H + Al, 0.3 mmolc dm⁻³ Ca, 1.0 mmolc dm⁻³ Mg, and 4 % of base saturation. Soil water content at field capacity (-0.01 MPa) was 180 g kg⁻¹ (Embrapa, 1997).

Dolomitic lime (28 % CaO, 20 % MgO and ECC 95 %) was applied to increase base saturation up to 70 % (Raij et al., 1996), and the soil was moistened to field capacity and placed in plastic bags for 30 days. Then, 150 mg dm⁻³ K (KCl) and 200 mg dm⁻³ P (simple superphosphate) were applied. Subsequently, 14 dm³ pots were filled with soil to reach the density of 1.2 g cm⁻³, so that similar field conditions would be needed. The pots were arranged in rows spaced 0.50 m apart, outside the greenhouse, for herbicide application, 47 days after planting. A non-selective systemic herbicide, Glyphosate, was applied at 1.0 kg ha⁻¹ (a.i.). The application was carried out with a CO₂ pressurized backpack sprayer with a spraying bar with four flat fan 110.04 nozzles (Spray Systems) spaced 0.40 m apart. Water volume and constant pressure for herbicide application were 40 mL m⁻² and 150 kPa, respectively. The bar was set 1.2 m above the soil surface and displacement speed was 1.0 m s⁻¹. Then, the pots were brought back to the greenhouse.

Plant shoots were collected on the 7th day after herbicide application, by cutting at soil level. Plants were fragmented into pieces of 3–5 cm. One day before rain simulation, sub samples were taken, weighed (fresh), dehydrated in a forced air oven for 24 h at 60 °C, and weighed again (dry) to calculate water content. The amount of fresh straw to be used in the rainfall simulation (8.0 Mg ha⁻¹) was calculated taking in account its water content. Part of the material was stored for N (sulfuric digestion and steam distillation) and C analysis (burning at 550 °C) as proposed by Embrapa (1997).

The rainfall simulator used was equipped with a bar supporting spraying nozzles, 1.45 m above the straw. Five high-flow nozzles (model TK-SS-20, Spray Systems) were used; nozzle spacing was 0.50 m. Traction in the spraying system was provided by belts and gears driven by electric motors, so that boom speed was maintained constant. The amount of rainfall applied at each boom passing was determined in previous tests, and the equipment was calibrated to apply a water volume of 2.5 mm. In other words, a 30 mm rainfall was simulated in 12 boom passings. The rainfall droplets produced by the simulator were generated at a pressure of 81 Pa, with a spray boom speed of 0.0925 m s⁻¹.

The residues of the four species were placed within rigid PVC tubes (area 0.049 m², height 0.10 m, and bottom of 10 mm-mesh nylon screen). Each ring was filled with the equivalent to 8.0 Mg ha⁻¹ dry matter. The tubes were fixed on plastic funnels, and the water, after passing through the residues, was collected in containers placed under plastic curtains (to avoid contamination by water from simulated rain). The structures containing the plant residues were placed at equidistant points along the boom travel course. One ring without residues was also included in all rainfall simulations to estimate N contents in the water. At the end of each rainfall application, the water that had passed through the residues was collected and filtered. The volume was determined and the water cool-stored (2 °C). Later, the amounts of N-NH₄⁺ and N-NO₃⁻ in the percolated water were determined by sequential distillation (Keeney & Nelson, 1982). After rainfall simulation, the straw was oven-dried at 60 °C for 72 h and N and C levels were determined.
The experiment design was a 4 x 4 factorial, arranged in completely randomized blocks, with four replications. Treatments consisted of four species grown under four N rates. The results were subjected to variance analysis. The t test (p ≥ 0.05) was applied to compare different species in each N rate. Regression analysis was used to evaluate the effects of N fertilization for each species. Significant equations with the highest coefficients of determination (F test, p ≥ 0.05) were selected.

RESULTS

The significance levels for shoot dry matter, N contents, N accumulation, C/N ratio, NH₄⁺ and NO₃⁻ washed from the straw of different cover grasses as related to N fertilization were obtained by ANOVA (F test) (Table 1).

There was a linear increase in pearl millet dry matter yields with N fertilization and responses were observed in Congo grass up to a rate of 155 kg ha⁻¹; both species are C₄. The highest N rate tripled pearl millet dry matter yield and increased Congo grass phytomass 1.8 times. For triticale, a C₃ grass, a quadratic equation was fitted and the highest dry matter yield was observed with 137 mg dm⁻³ N. Dry matter production of black oat was not increased with N rates (Figure 1).

Although pearl millet phytomass increased linearly with fertilizer rates (Figure 1), there was just a slight increase in tissue N concentrations at the highest N rate (Figure 2). The results, however, can be explained by a "dilution effect", since dry matter production of this crop was significantly increased (Figure 1). At the highest N rate the dry matter production of Congo grass was lower than of millet (Figure 1). Nevertheless, N concentrations in Congo grass plant tissue increased linearly with N fertilization up to 200 mg dm⁻³ (Figure 2). Therefore, considering only the C₄ plants, pearl millet dry matter and N concentrations in Congo grass tissue increased with increasing N rates.

Black oat dry matter yields were not affected by N fertilization (Figure 1), but a quadratic response in plant tissue to N concentrations was observed (Figure 2), while N concentrations increased linearly in triticale.

![Figure 1. Shoot dry matter of cover grasses as related to N fertilization, 54 days after sowing. Vertical bar show the LSD comparing species (p ≥≥≥≥≥ 0.05). ** R² significant (p ≤≤≤≤≤ 0.001); ns R² non significant.](image)

![Figure 2. Nitrogen level in shoot dry matter of different cover grasses, as related to N fertilization, 54 days after sowing. Vertical bar show the LSD comparing species (p ≥≥≥≥≥ 0.05). ** R² significant (p ≤≤≤≤≤ 0.001); ns R² non significant.](image)

Table 1. Significance levels (F test) for shoot dry matter, N contents, N accumulation, C/N ratio, NH₄⁺ and NO₃⁻ washed from the straw of different cover grasses as related to N fertilization

| Cause of variation | Dry matter | N content | N accumulation | C/N ratio | NH₄⁺ | NO₃⁻ |
|--------------------|------------|-----------|----------------|-----------|------|------|
| Species            | 0.001      | 0.001     | 0.001          | 0.001     | 0.001| 0.001|
| N rate             | 0.001      | 0.001     | 0.001          | 0.001     | 0.001| 0.001|
| Species x rate     | 0.001      | 0.001     | 0.001          | 0.160     | 0.402| 0.001|
Under the simulated 30 mm rainfall, the amount of NH$_4^+$ washed from the C$_3$ plants, black oat and triticale was higher than from the C$_4$ species Congo grass and pearl millet (Figure 3a). The amount of NH$_4^+$ leached from triticale residues increased linearly with N rates, but NH$_4^+$ leaching from black oat straw increased only up to 152 mg dm$^{-3}$ N. Nevertheless, the highest amount of NH$_4^+$ was leached from black oat residues. In spite of a significant linear response for Congo grass, rain water washed very little NH$_4^+$ from pearl millet and Congo grass residues.

Black oat was the only crop with higher NO$_3^-$ leaching at higher N rates (Figure 3b). At high N availability, N absorption may have been high enough to bring Nitrate Reductase activity to saturation, and eventually plant tissues accumulated nitrogen as free NO$_3^-$.

The total N accumulated in pearl millet, Congo grass and triticale plants increased linearly with N fertilization, whereas the response of N accumulated in black oat was quadratic (Figure 4). Hence, the tropical grasses Congo grass and millet were more efficient in extracting N from soil, although N levels in plant tissues were lower for these two crops than for black oat and triticale (Figure 2).

Pearl millet and Congo grass (C$_4$), had higher C/N rates than black oat and triticale (C$_3$), but N fertilization decreased the C/N ratios of all cover grasses (Figure 5).

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Figure 3. N-NH$_4^+$ (a) and NO$_3^-$ (b) leaching from cover crop plant residues after simulation of 30 mm rainfall as related to N fertilization. Vertical bar show LSD comparing species ($p \geq 0.05$). ** $R^2$ significant ($p \leq 0.001$); ns $R^2$ non significant.

Figure 4. N accumulated in shoot dry matter of different cover grasses as related to N fertilization, 54 days after sowing. Vertical bar show LSD comparing species ($p \geq 0.05$). ** $R^2$ significant ($p \leq 0.001$); ns $R^2$ non significant.

Figure 5. C/N ratio of the shoot dry matter of different cover grasses as related to N fertilization. Vertical bar show LSD comparing species ($p \geq 0.05$). ** $R^2$ significant ($p \leq 0.001$); ns $R^2$ non significant.
DISCUSSION

By modifying dry matter yields, N accumulation or the C/N ratio, N fertilization in plants with different photosynthetic pathways for C assimilation may affect N cycling and residue accumulation on the soil surface differently for the subsequent crop. Despite the fact that grasses are generally more N-demanding (Cantarella et al., 1997), tropical C4 species such as sugar cane, maize, sorghum, Congo grass, pearl millet, etc., are more efficient in using applied N for dry matter production and respond better to higher levels of applied N than temperate C3 grasses, e.g., wheat, triticale, ryegrass, rye, black oat, etc (Lazenby, 1981). In general, increases in dry matter yields in the C3 species were lower in our experiment (Figure 1), but N concentrations in plant tissues were higher than in Congo grass and millet, in response to applied N. According to Pimentel (1998), in C3 species such as black oat and triticale, N demand is high for the formation of the Rubisco enzyme, which accounts for about 50 % of the total amount of soluble proteins in leaves, generally resulting in higher N levels in leaf tissues and lower C/N ratios in plant residues. On the other hand, C4 species are more efficient in CO2 fixation and consequently, N is highly available for the formation of new tissues. Therefore, N levels usually decrease in the phytomass of these species, which is known as “dilution effect” (Pimentel, 1998).

In this study, lower N concentrations were found in Congo grass and millet residues, with a slight increase in leaf N, probably due to a higher amount of straw produced per unit of N applied (Figures 1 and 2). Rosolem et al. (2005) reported NH$_4^+$ leaching from six cover crops (black oat, millet, triticale, sann hemp, Congo grass, and sorghum) varying from 2.5 to 9.5 kg ha$^{-1}$, depending on rainfall, but without biological decomposition of the residues. The authors classified the species into two distinct groups: C3 plants (triticale, black oat and Indian hemp), with higher NH$_4^+$ leaching; and C4 grasses with lower NH$_4^+$ leaching. Findings by Rosolem et al. (2005) revealed that N was more readily leached by rain water from C3 than from C4 plant species per unit of phytomass. Results of the current study support the earlier findings of Rosolem et al. (2005). Plant tissue N concentrations were higher in C3 (black oat and triticale) than in C4 grasses (Congo grass and millet), regardless of the N fertilizer rates (Figure 2).

Physiological efficiency of C4 species is higher than of C3 plants, considering the low energy use to assimilate N in the former. These crops use less N to form enzymatic complexes for CO2 assimilation, and therefore, there is more N available for the growth and development of plant tissues in C4 plants (Marschner 1995; Pimentel 1998; Taiz & Zeiger 2002). This explains the higher response of Congo grass and millet to N fertilization (Figure 1). On the other hand, in the C3 species black oat and triticale large amounts of N were possibly consumed in the formation of the Rubisco enzyme and the capacity of C assimilation was also lower, resulting in reduced growth and eventually in lower N uptake. Hence, C4 crops were more efficient than C3 plants in N uptake and use.

Usually, plant residues can be classified in two groups; fast and slow decomposition types, i.e., residues with C/N ratios higher than 25 strongly immobilize N and have a low decomposition rate; and on the contrary, C/N ratios below 25 indicate high N mineralization rate and fast plant residue decomposition (Boer et al., 2007). Generally, the higher the N rate, the lower the C/N ratio observed in the plant residues, which decreased from 36 to 23, 35 to 19, 24 to 11 and 28 to 10 for millet, Congo grass, triticale and black oat, respectively. Despite the high capacity of C4 grasses of accumulating N in phytomass (Figure 4), the results of this experiment suggest that tropical species have a lower decay rate and immobilize greater amounts of N during decomposition, compared with temperate C3 crops.

Values of C/N ratio were inversely proportional to N levels (Figures 2 and 5). This indicates a C/N ratio decrease due to higher N levels in plant shoots as a result of higher N fertilization. In terms of plant residue management and crop rotation systems, Congo grass and pearl millet showed higher increases in phytomass and N accumulation at the highest N rate of 200 mg dm$^{-2}$ (Figures 1 and 4). However, N recycling to the soil in a system with these tropical C4 grasses may be delayed due to high C/N ratios (Figure 5); this could affect the nutrition of the subsequent crop grown in no till systems (Alta & Giacomini, 2003). Results for black oat and triticale differed, i.e., the highest N dose (200 mg dm$^{-2}$) for triticale and the calculated dose of 140 mg dm$^{-2}$ of N for black oat increased N content and decreased C/N ratios of the straw (Figures 2 and 5). In an experiment using litter bags, N release from Aamaranthus cruentus (C$_3$) was faster than from Pennisetum glaucum and Eleusine coracana (C$_4$), which confirms our results (Boer et al., 2007). Therefore, in systems where these C3 grasses are grown, at least part of the fertilizer N to be applied to the cash crop could be applied to the cover crops, which would recycle the N to the soil faster than C4 species. This could be an important strategy of preventing N losses from agricultural systems.

When choosing cover crop species preceding the main crop it is important to consider the low N recycling observed since the N release from cover crop residues should be synchronized with the subsequent crop demand. In some cases, C$_4$ grasses may be preferred in view of their ability to release N leaching at faster rates to the soil (Figure 3a). When cover crops are grown aiming at high amounts of phytomass, C$_4$ species should be chosen, as they produce higher straw yield, irrespective of N fertilization (Figure 1).
CONCLUSIONS

1. C₄ species accumulate more dry matter and nitrogen than C₃ species, but the nutrient is less washed by rains.

2. Under conditions where N losses and cycling are important, C₃ species might be a better option.

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