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Contrasting impacts of grass species on nitrogen cycling in a grazed Sudanian savanna

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ABSTRACT

We investigated the impact of perennial and annuals grass species on nitrogen cycling in a Sudanian savanna of Burkina Faso. We also analysed how the local context in terms of grazing and soil properties modifies these impacts. We selected four plots differing both by the intensity of grazing by cattle and soil depth, and used soil and grass biomass ^{15}N as integrative indicators of N cycle. If perennials are able to foster a more efficient nitrogen cycling there should be lower ^{15}N abundances in their biomass and soil. If soil depth and cattle pressure significantly modify nitrogen fluxes, soil depth and cattle pressure should influence ^{15}N signatures. Our results suggest that perennial grasses are more conservative for nitrogen (inhibition of nitrification, less leaching *via* a perennial root system, slower cycling). The increase in leaf $\delta^{15}N$ with N concentration is steeper in *Loudetia togoensis* than in the three other grasses. No significant difference was found between the ^{15}N signatures of the four plots. Our results on ^{15}N signatures and the fact that perennial grasses are much more abundant in the plots that are less grazed and have deeper soils, confirm that the switch from perennial to annual grasses is linked to a degradation in soil fertility and pasture quality. This suggests that ^{15}N signatures can be used as indicators of fertility.

1. Introduction

Plants influence nutrient cycling and their long term availability (Chapman et al., 2006; Hobbie, 1992), and this feedbacks on their own growth and primary productivity. This is achieved through many mechanisms (Hobbie, 1992; Knops et al., 2002). This allows them to cope with abiotic constraints of ecosystems such as the lack of mineral nutrients and contrasting soil types and with biotic constraints such as herbivory or competition between plants (Craine, 2009; Grime, 2001). Among all possible strategies, the distinction between perennial and annual plants is particularly relevant in terms of nitrogen cycle and competitive ability (Grime, 1977, 2001). Annuals are viewed as opportunistic. They need to acquire their resources as quickly as possible and benefit from the immediately available mineral resources. Perennials are more likely to influence local nutrient cycles and to benefit from such modifications because their root system, their root exudates and their litter (roots and leaves) interact for a longer period with the same patch of soil and soil micro-organisms living in this patch (Vinton and Burke, 1995). Perennials grow slower than annuals but may benefit from sparing soil mineral nutrient resources, from increasing the local availability of these resources and from decreasing nutrient losses. It has for example been shown in tropical savannas that many perennial bunch grasses are able to inhibit nitrification through the release of particular molecules from their root systems (Lata et al., 2004; Subbarao et al., 2007a, 2007b), which increases their own biomass (Boudsocq et al., 2009). As these grasses live for several tens of years, they build dense root systems where the close proximity between dead and live roots is likely to induce a very efficient nutrient recycling and reduce nitrogen losses (Abbadie and Lata, 2006; Abbadie et al.,

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1992). In this context, our primary goal was to compare, from the point of view of nitrogen cycling, two annual and two perennial bunch grasses growing in the same West African savanna.

To meet this goal, we measured the natural abundance in ^{15}N of the biomass of these four grasses and of the soil penetrated by their root system. Indeed, ^{15}N abundance is considered as a good indicator of ecosystem functioning (Dijkstra et al., 2008; Robinson, 2001; Staddon, 2004; Templer et al., 2007). First, ^{15}N abundance is used to determine the origin of soil organic matter (Boutton et al., 1998; Danso et al., 1993). Similarly, the ^{15}N of biomass gives hints on the origin of the nitrogen. For example legumes, due to symbiotic nitrogen fixation, tend to have lower concentrations in ^{15}N than other plants and thus to decrease soil ^{15}N concentration through their litter. Second, the isotopic fractionation due to the lower reactivity of ^{15}N relatively to ^{14}N allows tracing nitrogen fluxes in the soil. For example, rapid nitrogen cycling and nitrification are thought to increase ^{15}N abundance (Dijkstra et al., 2008), while tighter nitrogen cycling (e.g. less leaching and denitrification) should lower ^{15}N abundances. The difference between plant and soil ^{15}N abundances gives hints on plant preference for ammonium *vs*. nitrate (Kahmen et al., 2008). Similarly, the discrepancy between the ^{15}N signatures of the biomass of an African perennial bunch grass and bulk soil (lower ^{15}N in the biomass than in the soil organic matter) (Abbadie et al., 1992) suggested that this grass fosters an efficient nitrogen cycling through the recycling of the nitrogen contained in the roots of its dense and localized root system. Taken together, we predict that our two perennial and two annual grasses should have different ^{15}N signatures, which should in turn impact soil signatures. For the same reasons the universal positive correlation between leaf ^{15}N signature and leaf N concentration (Craine et al., 2009b) should also be different between annual and perennial biomasses.

Besides plant influence on nitrogen cycling, other factors such as herbivory and soil properties are likely to impact these cycles, which should in turn modify ^{15}N isotopic signatures. Cattle or wild herbivores indeed quicken carbon and nutrient cycling by increasing the turnover of the biomass. Herbivore impact on nutrient cycling has been extensively studied. On the one hand, an important issue is to determine whether herbivores could increase the efficiency of nitrogen cycling (decrease nitrogen losses), which could increase primary production on the long term (de Mazancourt et al., 1998). On the other hand, they have been shown to impact many fluxes of mineral nitrogen but the direction of these effects is likely to be case dependent (McNaughton et al., 1997; Wardle et al., 2001). For example, cattle have been shown to increase nitrification, denitrification and free nitrogen fixation in a temperate grassland (Patra et al., 2006). In turn, the modification of these fluxes impacts ^{15}N signatures and may either increase (Frank and Evans, 1997) or decrease (Frank et al., 2000) soil ^{15}N abundances. Similarly, soil properties such as soil depth, soil compaction, soil texture or soil organic matter content are likely to impact biomass and soil ^{15}N signatures (Abadín et al., 2010) through their effects on soil capacity to retain mineral nutrients, or through their effects on microbial biomass and activities.

Taking into account these interactive impacts of plant species and soil type/herbivores on nitrogen cycling and detecting these interactions using ^{15}N signatures is interesting in two contrasted ways. On the one hand, such complex interactions could strongly increase the variability in isotopic signatures, which could blur potential effects of targeted treatments (here the plant species). Documenting these interactions and their effects on isotopic signatures is thus methodologically useful to help designing and interpreting other experiments or field samplings. On the other hand, isotopic signatures might allow analysing the impact of herbivores and different soil types on nitrogen cycling and might help disentangling the interactions between herbivores or soil types and plant species.

For these reasons, we compared two annual and two perennial grasses in a West African savanna where both soil depth and cattle pressure are varying. Taken together, we make the following predictions. If perennials are able to foster a more efficient nitrogen cycling, (1) there should be lower ^{15}N abundances in their biomass and soil and (2) the relation between ^{15}N signature and N concentration should be different between annual and perennial leaf biomass. If soil depth and cattle pressure significantly modify nitrogen fluxes, (3) soil depth and cattle pressure should influence ^{15}N signatures, (4) plant species and soil depth or cattle pressure should affect ^{15}N signatures in an interactive way, i.e. the impact of each species should depend on soil depth and cattle pressure.

2. Material and methods

2.1. Study site

The study site is the third management unit of the protected forest of Dindéresso (FCD) in the West of the town of Bobo-Dioulasso, at the altitude of 390 m (11°12.494′ north, 4°24.159′ west). The climate is South-Sudanian: there is a wet season from May to October and a dry season from November to April. The area is located between the 900 and 1250 mm isohyets. 1254 mm of rain fell in 2010 but only 831 mm in 2011. The mean annual temperature is 28 °C. The whole forest lies on sedimentary rocks and our own study area lies on Bobo-Dioulasso sandstone. Soils are tropical ferruginous leached soils indurated or not. Vegetation consists in a shrub savanna grazed by cattle. It is characterized by the following dominant shrub/small tree species: *Vitellaria paradoxa*, *Terminalia laxifolia*, *Detarium microcarpa*, *Parkia biglobosa*, *Guiera senegalensis*, *Combretum nigricans*, *Gardenia ternifolia*. The herbaceous layer is dominated by grasses. The main annual grasses are *Andropogon pseudapricus*, *Loudetia togoensis*, *Microchloa indica*. The main perennial grasses are *Andropogon gayanus*, *Andropogon ascinodis*, *Hyparrhenia subplumosa*, *Schizachyrium sanguineum* and they are all bunch grasses. There are some legumes (*Cassia mimosoides*, *Indigofera trichopoda*, *Zornia glochidiata*, *Tephrosia pedicelata*, *Tephrosia bracteolate*), Cyperaceae (*Fimbristylis hispidula*) and other forbs (*Waltheria indica*, *Pandiaka heudelotii*, *Spermacoce stachydea*, *Striga hermonthica*). Grass aboveground biomass and necromass are burnt each year by bushfires.

The study site has been divided in four blocs of approximately 1.5 ha according to the dominance of perennial and annual grasses and the frequency of grazing: blocs 1 and 2 are dominated by annual grasses and are more grazed by cattle during the rainy season while blocs 3 and 4 are dominated by perennial grasses and are less grazed during the rainy season. Blocs 1 and 2 are indeed next to the main road, so that they are more easily reached by cattle and shepherds, and blocs 3 and 4 are supposed to host an abundant population of tsetse flies during the rainy season so that they are avoided to limit the risk of cattle infection by trypanosomiasis. Blocs 1 and 2 have shallower soils (at most 55 cm deep, they are indurated ferruginous leached soils). Blocs 3 and 4 have deeper soils (at least 105 cm deep, the indurated layer is deeper). Blocs 1 and 2 are contiguous and so are blocs 3 and 4. Blocs 1–2 and 3–4 are separated by a distance of about 2000 m.

2.2. Soil and biomass sampling

The study focusses on four of the dominant grass species: *Andropogon pseudapricus*, *Loudetia togoensis* (annuals), *Andropogon ascinodis* and *Andropogon gayanus* (perennial bunch grasses). The four grasses are cespitosus but the tussocks of the two perennials

are much larger than the ones of the two annuals. The four species have C_4 photosynthesis (Breman and De Ridder, 1991). As a control, bare soils were also sampled. In each of the four blocs, 2 or 3 replicates of each plant species or bare soil areas were randomly selected: an individual for perennial bunch grasses, a small patch covered of individuals of the same species for annuals and a small patch deprived of plants for bare soils. In total, 10 replicates were sampled for each species and bare soils and they were spread over the 4 blocs. Taken together, 50 sampling units (plant species or bare soil) were investigated. In each sampling unit a soil sample was collected in June 2011 from the 0–10 cm layer using a core auger (5 cm diameter). This sample was taken in the middle of tussocks for perennial grasses. Soil samples were then air dried in the shade and sieved at 2 mm. Leaves and roots were collected from each annual or perennial grass sampling unit. Roots were collected using a core auger (5 cm diameter) and washed with water. Leaves and roots were oven-dried at 70 °C for 72 h. In total, 40 samples of roots, leaves and soil were analysed as well as 50 soil samples (40 samples for annual and perennial grasses and 10 for bare soils).

2.3. Soil and biomass analyses

Soil and biomass samples were thinly ground and their content in N, C, ^{15}N measured by EA-IRMS (Carlo-Erba NA-1500 NC Elemental Analyser on line with a Fisons Optima Isotope Ratio Mass Spectrometer). As usually done (Wang et al., 2010), the contents in ^{15}N was expressed as relative differences in the ratios $^{15}N/^{14}N$ between samples and international standards:

$$\delta^{15}N(‰) = \left(\left(^{15}N/^{14}N\right)_{sample} \Big/ \left(^{15}N/^{14}N\right)_{standard} - 1\right) 1000,$$

The international standard for N is the atmospheric N_2.

2.4. Statistics

All variables were analysed using ANOVAs testing for the effect of the bloc, the grass species and the interaction between the two (Tables 1 and 2). When the interaction was not significant it was removed from the model. When only simple effects were significant the direction of their effects was determined using the estimated model parameters and post-hoc Tukey tests. When the interaction was significant the variable was reanalysed separately for each grass species (or bare soil). Graphs (Figs. 1–2) only display results for factors and factor combinations that are significant. The relation between biomass $\delta^{15}N$ and N concentration was studied using ANCOVAs testing for the effect of N concentration, the effect a categorical variable (root vs. leaves or the grass species) and the interaction between the two. All these analyses were achieved using R software (R development core team, 2010).

Table 1
Analyses of variance of soil N concentration and $\delta^{15}N$ and C concentration. The effect of the bloc, the grass species and the interaction between the two are tested. F values are given together with an indication of significance. The last line indicates the direction of effects through the ordering of treatments. When the interaction is significant this line gives the results of ANOVAs analysing separately the bloc effect for each species.**, P < 0.01; ***, P < 0.001; ns, no significant effect; df, degree of freedom; 1, 2, 3, 4, blocs; AA, *A. ascinodis*; AG, *A. gayanus*; AP, *A. pseudapricus*; LT, *L. togoensis*; BS, bare soil.

	Df	Soil $\delta^{15}N$	Soil N	Soil C
Bloc	3	1.24	2.14	0.61
Species	4	4.34**	0.57	1.04
BlocXSpecies	12	–	2.68*	2.09**
R^2		0.33	0.58	0.57
Direction of effects		AP, LT, BS > AA, AG	In AA: 1, 3 >2, 4 In AG: ns In AP: 3, 4 >1, 2 In LT: 1, 4 >2, 3 In BS: ns	In AA: ns In AG: ns In AP: 3, 4 > 1, 2 In LT: 1, 4 > 2, 3 In BS: ns

Table 2
Analyses of variance of root and leaf N concentration and $\delta^{15}N$. The effect of the bloc, the grass species and the interaction between the two were tested. The interaction was never significant and was thus removed from the model. F values are given together with an indication of significance. The last line indicates the direction of effects through the ordering of treatments. When the interaction is significant this line gives the results of ANOVAs analysing separately the bloc effect for each species.**, P < 0.01; ***, P < 0.001; ns, no significant effect; df, degree of freedom; 1, 2, 3, 4, blocs; AA, *A. ascinodis*; AG, *A. gayanus*; AP, *A. pseudapricus*; LT, *L. togoensis*; BS, bare soil.

	Df	Root $\delta^{15}N$	Root N	Leaf $\delta^{15}N$	Leaf N
Bloc	3	3.75*	14.10***	4.70**	3.10*
Species	3	13.88*	44.92***	8.79***	16.63***
R^2		0.74	0.88	0.73	0.70
Direction of effects		AP, LT >AA, AG 1 > 3, 4	AP, LT >AA, AG 1, 2, 3 > 4 2 > 3	AP >LT, AA, AG 1, 2 > 4	AP >LT, AA, AG 2, 3 > 4

3. Results

Soil $\delta^{15}N$ is significantly lower (Table 1 and Fig. 1) for the two perennial species (respectively 3.98 and 3.88‰ for *A. ascinodis* and *A. gayanus*, see Table 3 for all values) than for the two annual species and the bare soil (respectively 4.38, 4.55 and 4.56‰ for *A. pseudapricus* and *L. togoensis* and bare soil), but is not affected by the bloc. This corresponds to a decrease in $\delta^{15}N$ of about 0.57‰.

Soil N concentration is affected by the interaction between the grass species and the bloc: under *A. ascinodis* N concentration is higher in blocs 1 and 3 than in blocs 2 and 4, under *A. pseudapricus* it is higher in blocs 3 and 4 than in blocs 1 and 2, and under *L. togoensis* it is higher in blocs 1 and 4 than in blocs 2 and 3. Soil C concentration is affected by the interaction between grass species and the bloc (Fig. 2, Table 1): under *A. pseudapricus* it is higher in blocs 3 and 4 than in blocs 1 and 2, under *L. togoensis* it is higher in blocs 1 and 4 than in blocs 2 and 3.

Biomass $\delta^{15}N$ is overall higher under the two annual than under the two perennial grasses (Fig .1 and Table 2). Both for roots and leaves there is a significant effect of species and the bloc on biomass $\delta^{15}N$ but the interaction between these two factors is not significant (Table 2). For roots, the $\delta^{15}N$ is higher (Fig. 2) in the two annuals (respectively 2.60 and 1.26‰ for *A. pseudapricus* and *L. togoensis*) than in the two perennials (respectively −0.72 and −0.99‰ for *A. ascinodis* and *A. gayanus*) and in bloc 1 than in blocs 3 and 4 (respectively 1.44, -0.17 and −0.09‰ for blocs 1, 3 and 4). This corresponds to an increase in $\delta^{15}N$ of about 2.8‰ between perennials and annuals. For leaves, the $\delta^{15}N$ is higher (Fig. 2) in *A. pseudapricus* (2.74) than in the three other species (respectively 0.67, -0.59 and −0.30‰ for *L. togoensis*, *A. ascinodis* and *A. gayanus*) and in blocs 1 and 2 than in bloc 4 (respectively 1.74, 1.18 and −0.53‰ for blocs 1, 3 and 4). Overall, the results on soil and biomass $\delta^{15}N$ support our hypothesis that perennial grasses lead to lower $\delta^{15}N$ than annuals (first hypothesis in the Introduction). However, these results are at odds with our third and fourth hypothesis.

Both for roots and leaves there is a significant effect of species and the bloc on the biomass N concentration but the interaction between these two factors is not significant (Table 2). Root N concentration is higher (Fig. 2) in the two annuals (respectively 4.09 and 3.65 mg g^{-1} for *A. pseudapricus* and *L. togoensis*) than in the two perennials (respectively 2.52 and 3.71 mg g^{-1} for *A. ascinodis* and *A. gayanus*) and in blocs 1, 2, 3 (respectively 3.39, 3.72 and

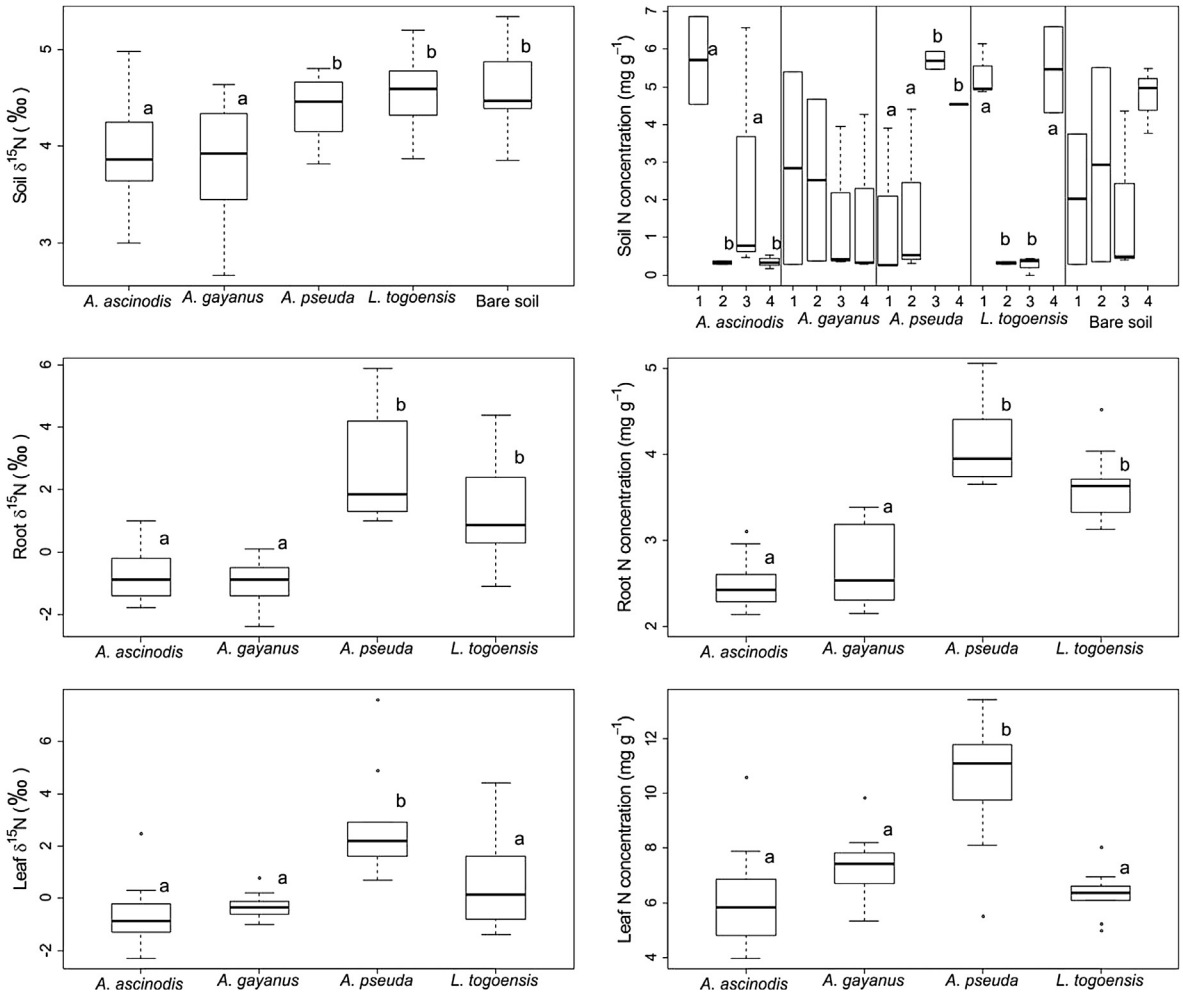

Fig. 1. Boxplots for N content and $\delta^{15}N$ of the soil, the roots and the leaves as a function of blocs and grass species. Medians are displayed together with 25th and 75th quartiles. Treatments and combinations of treatments that are displayed correspond to significant effects as determined by the ANOVAs (Table 1). Different letters denote significant differences between treatments. When the interaction between bloc and grass species is significant different letters denote differences between blocs within a grass species. 1, 2, 3, 4, blocs; *A. pseuda* stands for *A. pseudapricus*.

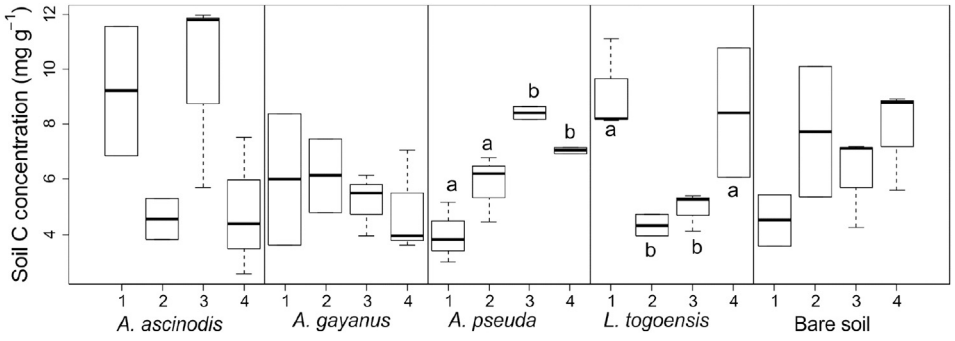

Fig. 2. Boxplots for soil C concentration. Medians are displayed together with 25th and 75th quartiles. Treatments and combinations of treatments that are displayed correspond to significant effects as determined by the ANOVAs (Table 2). Different letters denote significant differences between treatments. When the interaction between bloc and grass species is significant different letters denote differences between blocs within a grass species. 1, 2, 3, 4, blocs; *A. pseuda* stands for *A. pseudapricus*.

3.13 mg g^{-1}) than in bloc 4 (2.77 mg g^{-1}) and higher in bloc 2 than in bloc 3. This corresponds to an increase in N root concentration of about 48% between perennials and annuals. Leaf N concentration, as $\delta^{15}N$, is higher (Fig. 2) in *A. pseudapricus* (10.55 mg g^{-1}) than in the three other species (respectively 6.32, 6.20 and 7.41 mg g^{-1} for *L. togoensis*, *A. ascinodis* and *A. gayanus*) and in blocs 2 and 3 than in bloc 4 (respectively 8.24, 8.26 and 6.41 mg g^{-1} for blocs 2, 3 and 4).

A first ANCOVA shows that there is an overall positive effect of the biomass N concentration on the $\delta^{15}N$ and that the slopes of the relation between the two variables is steeper for roots than for leaves (Fig. 3). A second ANCOVA shows that for roots, the relation is the same for all species ($\delta^{15}N = 1.95\ N_R - 5.64$, where N_R is the

Table 3
Means and standard deviations (in parentheses) for biomass and soil N concentrations (mg g^{-1}) and $\delta^{15}N$ (‰).

	Soil $\delta^{15}N$	Soil N	Root $\delta^{15}N$	Root N	Leaf $\delta^{15}N$	Leaf N
A. ascinodis	3.98 (0.55)	2.09 (2.76)	−0.72 (0.86)	2.52 (0.31)	−0.59 (1.32)	6.20 (1.95)
A. gayanus	3.88 (0.60)	2.03 (2.21)	−0.99 (0.73)	2.71 (0.47)	−0.30 (0.52)	7.41 (1.17)
A. pseudapricus	4.38 (0.34)	3.01 (2.37)	2.60 (1.78)	4.09 (0.45)	2.74 (2.06)	10.55 (2.33)
L. togoensis	4.55 (0.43)	2.84 (2.75)	1.26 (1.80)	3.65 (0.41)	0.67 (1.92)	6.32 (0.85)
Bare soil	4.56 (0.44)	2.94 (2.27)	–	–	–	–

root biomass N concentration). A third ANCOVA shows that for leaves the relation varies significantly between species (Fig. 3): the slope is steeper for *L. togoensis* ($\delta^{15}N = 1.33\ N_L - 7.74$, where N_L is the leaf biomass N concentration) than for the three other species ($\delta^{15}N = 0.50\ N_L - 3.78$). These results only partially support our second hypothesis (see the Introduction). We also checked that the bloc does not influence the relation between $\delta^{15}N$ and N concentration in roots and leaves.

4. Discussion

Our results support, at least partially, two of the hypotheses (the first and the second) we set in our introduction: (1) ^{15}N signatures suggest that perennial and annual grasses do not have the same impact on nitrogen cycling. (2) The increase in leaf $\delta^{15}N$ with N concentration is steeper in *L. togoensis* than in the three other grasses. (3) ^{15}N signatures do not allow to point at any overall effect (whatever the grass species) of cattle grazing or soil depth on grass functioning or nitrogen cycling. (4) ^{15}N signatures do not suggest any interaction between grass species and blocs that would affect nitrogen cycling.

4.1. Impact of grasses on $\delta^{15}N$

The $\delta^{15}N$ values are higher in the soil of annual grasses and bare soil than in the soil of perennial grasses and, consistently, the same qualitative pattern is found for the $\delta^{15}N$ of the grass biomasses. Since the source of nitrogen should be overall the same for plants growing next to each other, this pattern must be due to fine scale differences between species in the various nitrogen fluxes leading to isotopic fractionation. The observed higher soil $\delta^{15}N$ under annual grasses and in bare soil can be interpreted as an indicator of increased rates in nitrogen cycling (mineralization, nitrification and denitrification) that also foster nitrogen losses (Nacro et al., 2004; Templer et al., 2007, 2008). Indeed, fractionation likely leads to an enrichment of the residual pool in ^{15}N because ^{14}N, the lighter of the two stable isotope, is more reactive (Mariotti et al., 1981). Thus, in the case of increased rates of nitrogen cycling, ^{14}N is more likely to leave the ecosystem through denitrification and leaching, which leads to an increase in soil $\delta^{15}N$.

This interpretation is fully compatible with a previous study (Abbadie et al., 1992) that compared the ^{15}N signature of soil below perennial bunch grasses (+3.4‰) and between these grasses, i.e. a bare soil situation (about +5‰). Here, we found qualitatively the same difference (+4.0‰ for perennials vs. +4.5‰ for annuals and bare soils) that can be interpreted as in Abbadie et al. (1992). There are differences in nitrogen fluxes and fractionation between the soil below perennial grass tufts and the soil between these tufts or below annuals grasses. These differences allow differences in ^{15}N signatures to build up slowly along the several tens of years of the life of these perennials. Indeed, the soil below grass tufts gets slightly impoverished in ^{15}N (relatively to the soil between tufts or below annuals), grasses take up this nitrogen so that the biomass of their root also gets impoverished in ^{15}N, some roots die and release mineral nitrogen impoverished in ^{15}N, differences in fractionation further decrease the ^{15}N signature of the nitrogen that can be absorbed by other roots of the same grass tuft, and so on….

Other studies have shown that soil and biomass $\delta^{15}N$ values depend on plant species (Kahmen et al., 2008; Kriszan et al., 2009; Nadelhoffer et al., 1996; Templer et al., 2005; Wang et al., 2010). As hypothesized in the introduction, we suggest that perennial grasses are able to influence the nitrogen cycle in a way that increases the availability of nitrogen in their favour. The fact that $\delta^{15}N$ values of bare soils are identical to the values of annual grasses supports this rationale. Several mechanisms leading to a lower fractionation below perennial bunch grasses than between these grasses and below annuals are probably leading to this general pattern: (1) Many African perennial bunch grasses (e.g. *Hyparrhenia* sp., *Brachiaria sp.*) have been shown to inhibit nitrification (Lata et al., 2004; Subbarao et al., 2007a, 2007b). Perennial grasses are able to build a dense and perennial root system so that (2) the proximity between dead and living roots reduces the risk of leaching of the mineral nitrogen released by root decomposition (Abbadie and Lata, 2006; Abbadie et al., 1992) and (3) rates of leaching are likely to be further decreased by the fact that living roots are always present and able to take up available mineral nutrients (Joffre, 1990). Testing fully our hypotheses and interpreting thoroughly the ^{15}N signatures will off course require (1) documenting precisely nitrogen fluxes such as nitrification and denitrification potentials, (2) measuring the ^{15}N signatures of all nitrogen sources, (3) taking into account mechanisms that could complicate the interpretation of ^{15}N signatures such as nitrogen fixation by free bacteria or endophytes (Elbeltagy et al., 2001).

4.2. Impact of soil and herbivores on $\delta^{15}N$

Herbivores (Aranibar et al., 2008; Craine et al., 2009a; Frank and Evans, 1997; Frank et al., 2000) and soil properties (Abadín et al., 2010) have often been shown to impact soil $\delta^{15}N$, which is an indication of modifications of nitrogen cycling. In particular, herbivores impact nitrogen cycling and aboveground–belowground linkages both directly through changes in plant growth and physiology and alterations of the quality and quantity of mineral and organic resources and, indirectly, through changes in the functional composition of vegetation (Bardgett and Wardle, 2003). Here, the species-specific $\delta^{15}N$ of the soil does not depend on the four blocs while root and leaf $\delta^{15}N$ tends to be higher in the blocs with the higher cattle pressure (and shallower soil). This suggests (1) that increasing cattle pressure and decreasing soil depth has a relatively weak direct effect on nitrogen cycling and/or that this effect is recent (effects on biomass $\delta^{15}N$ but no effect on soil $\delta^{15}N$), (2) that this effect tends to increase the rate of nitrogen cycling and nitrogen losses (Dijkstra et al., 2008; Templer et al., 2007).

Besides, our sampling design is based on measurements made at the scale of individual tufts for perennial grasses and small monospecific patches for annual grasses and we investigated equally the same four grass species in the four blocks. In fact, grazing pressure and soil depth do not change the impact of each grass species on soil $\delta^{15}N$ but these factors likely strongly impact nitrogen cycling and ^{15}N signatures, indirectly, through their effects on vegetation.

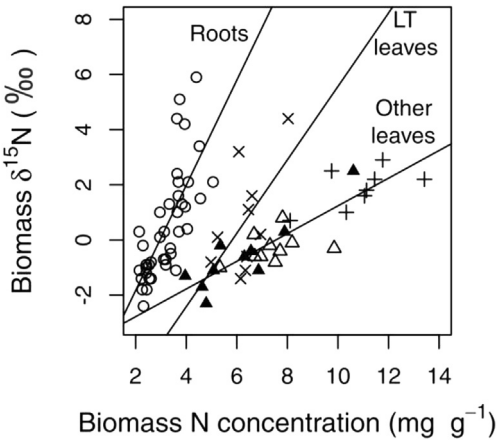

Fig. 3. Regression between biomass δ^{15}N and N concentrations. The regression is significantly different between roots and leaves, and within leaves between *L. togoensis* (LT leaves) and the 3 other species (Other leaves). Circles, roots of the four species; crosses, *L. togoensis* leaves; plus, *A. pseudapricus* leaves; filled triangle, *A. ascinodis* leaves; triangle, *A. gayanus* leaves.

Perennial grasses are dominant in the two blocs with deeper soils and lower grazing pressures, while annual grasses are dominant in the two other blocs. This would be the hint that cattle and shallow soils slow down or even reverse the normal succession, which is one of the main mechanisms through which herbivores impact ecosystem functioning (Bardgett and Wardle, 2003). Indeed, annuals normally start to grow in fallows and are progressively replaced by perennials (Bilgo et al., 2006; Somé, 1996). Thus, overall, at the scale of the whole savanna lower soil depth and higher cattle pressure lead to higher δ^{15}N values through a switch in the dominant grass species (see the first section of the Discussion). Note that the direct effects of cattle pressure also lead to an increase in δ^{15}N, at least in biomasses. This is overall a mark of a faster nitrogen cycling and higher nitrogen losses. This is in line with published results (Frank and Evans, 1997; Patra et al., 2006) emphasizing cases of negative effects of herbivores on soil fertility. This also suggests that our blocs 1 and 2 are overgrazed while a milder grazing pressure (blocs 3 and 4) do not lead to such negative effects both in terms of (1) the switch form perennial to annuals grasses and (2) the long-term soil fertility.

We also predicted that the interaction between grazing intensity and grass species should impact nutrient cycling because species-specific effects on nitrogen cycling could be impacted by grazing, e.g. through differences in palatability. For example, perennial grasses could control efficiently nitrogen cycling and limit nitrogen losses only when they are not grazed intensively and have high biomasses. The absence of significant interactions between bloc and grass species for soil and biomass δ^{15}N further supports the idea that at a small scale the impact of each grass species and bare soil drives the local nitrogen cycle and not grazing or soil depth.

4.3. Impacts of grasses, soil type and herbivory on nitrogen concentrations

The more efficient nitrogen cycling fostered by perennial than by annual grasses and suggested by δ^{15}N values could have led to higher soil nitrogen concentrations for perennials than for annuals and bare soils. Perennial grasses have indeed been found to increase nitrogen content within their root systems (Jackson and Caldwell, 1993; Vinton and Burke, 1995). Such a pattern was not found, and instead, soil nitrogen content depends on a complex interaction between plant species and bloc. This pattern is difficult to explain in details. It seems that the likely interactions between plant species impact on nutrient cycling and grazing by cattle or soil depth is more influential for soil N concentration than for soil or biomass δ^{15}N. The pattern must be linked to the fact that the likely reduced rate of nitrogen losses under perennials does not necessarily lead to the buildup of the nitrogen stock within the soil because perennial grasses also store a high amount of nitrogen in their root and shoot biomass. Such an apparent discrepancy between fluxes and stocks was observed in Lamto savanna in Ivory Coast where subpopulations of perennial grasses controlling or not nitrification lead to 2-fold differences in grass biomass but not in soil N content (Lata et al., 1999). Our results on soil carbon concentration confirms the idea that in this savanna perennial grasses improve their own access to nitrogen but do not necessarily increase locally soil C or N concentrations.

The pattern of nitrogen concentration within grass biomass is simpler: in leaves the nitrogen concentration is higher in *A. pseudapricus* biomass than in the three other species, in roots the nitrogen concentration is higher in the two annuals than in the two perennials. An explanation could be that annual grasses tend to have higher nutrient concentrations than perennials because they favour a quick growth (Craine et al., 2012; Garnier and Vancaeyzeele, 1994). This is supported by our root results but only partially by our leaf results: only one annual (*A. pseudapricus*) has a higher leaf N concentration than the two perennials. Nitrogen concentration depends on the availability of mineral nitrogen but is also linked to plant ecophysiological traits such as the capacity to take up nitrogen or nitrogen use efficiency but we so far do not have a clear explanation for the higher value observed for *A. pseudapricus* leaves.

4.4. Relation between δ^{15}N and N concentration in biomasses

Our results are consistent with the already described worldwide positive correlation between δ^{15}N and N concentration in leaf biomasses (Craine et al., 2009b). In comparison to a global data base, our leaf N concentrations are rather low (below 12 mg g^{-1} in most cases) and our leaf δ^{15}N values are intermediate (between −2 and 4‰ in most cases). Our leaf δ^{15} values are relatively high taking into account our rather low N concentration values (see Fig. 3c in Craine et al., 2009b). These values are compatible with the global increase in δ^{15}N with the mean annual temperature (our study site has a high mean temperature, about 28 °C) and the global decrease with the mean annual precipitation (our study site has an intermediate mean annual precipitation, about 1000 mm yr^{-1}) (Craine et al., 2009b).

We expected different slopes for the relation between leaf N concentration and δ^{15}N between annuals and perennials. Somehow, a species for which N concentration increases quicker with δ^{15}N is able to allocate more nitrogen to its leaves for a given rate of nitrogen cycling and a given rate of openness of this cycling (Dijkstra et al., 2008; Templer et al., 2007). This species would be more efficient in terms of exploitation of nitrogen than species with N concentrations increasing slower with δ^{15}N. We did find a steeper relation between N concentration and δ^{15}N for the annual species *L. togoensis*. However, we found the same relation for the other annual and the two perennials. All factors influencing the δ^{15}N, e.g. mycorrhization and the type of mycorrhiza (Craine et al., 2009b), could explain this difference between *L. togoensis* and the three other species. More information would be needed to interpret this result.

Our results on the relation between root N concentration and root δ^{15}N are consistent with the comparison of 90 grass species across four regions of the world (Craine et al., 2005). This relation

parallels the relation between leaf N concentration and leaf $\delta^{15}N$ but roots have lower N concentrations than leaves so that the $\delta^{15}N$ increases more steeply with N concentration for roots than for leaves. For roots the relation between N concentration and $\delta^{15}N$ is the same for the four species while, for leaves, *L. togoensis* displays a particular relation. This suggests that the particular strategy of this species for N management aims at increasing leaf N concentration and photosynthesis and not at improving the acquisition of soil resources.

5. Conclusion

Our main conclusion remains that perennial grasses decrease soil and biomass ^{15}N signatures relative to annual grasses and bare soil. This confirms the usefulness of ^{15}N as an integrative tool to assess nitrogen cycling (Kahmen et al., 2008; Templer et al., 2007). This suggests that perennials better control nitrogen cycling through a slower cycling and lower rates of nitrogen losses through leaching and denitrification: perennial grasses would have evolved particular mechanisms to improve nitrogen cycling in their favour, which would ultimately feedback on their growth allowing them to accumulate more biomass. These arguments are further supported by the fact that annual grasses can be viewed as *r* or ruderal species in comparison to perennial grasses (Grime, 1977, 2001). In annuals, evolution should have selected strategies that favour the immediate use of mineral resources. Perennials, on the contrary, should benefit from strategies allowing them to make the best use of mineral resources on the long term, which should lead to lower rates of nutrient cycling and lower rates of nutrient losses.

The mechanisms evolved by perennials could be involved in the high primary productivity of Guinean humid savannas (Boudsocq et al., 2009; Gignoux et al., 2006) and could increase the primary productivity of Sudanian savannas dominated by perennial grasses (for example, here in Burkina Faso). In turn, our results are consistent with the idea that annual grasses are a mark of soil and vegetation degradation, and that annual grasses tend to replace perennials in less fertile soil conditions and after over-grazing (Belsky and Blumenthal, 1997; Burke et al., 1998; César, 1989; Derner et al., 1997; Rossignol et al., 2006). Here, because the higher cattle pressure occurs in the area of shallower soils it was *a priori* not possible to disentangle their possible effects, which should be possible with an experimental approach excluding cattle from some patches. Anyway, our results suggest that soil or biomass ^{15}N signatures could be used as a diagnostic tool for the degradation of pastures and the fertility of their soils.

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