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

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15

16 **ABSTRACT**

17 We investigated the impact of perennial and annuals grass species on nitrogen cycling in a
18 Sudanian savanna of Burkina Faso. We also analyzed how the local context in terms of
19 grazing and soil properties modifies these impacts. We selected four plots differing both by
20 the intensity of grazing by cattle and soil depth, and used soil and grass biomass ¹⁵N as
21 integrative indicators of N cycle. If perennials are able to foster a more efficient nitrogen
22 cycling there should be lower ¹⁵N abundances in their biomass and soil. If soil depth and
23 cattle pressure significantly modify nitrogen fluxes, soil depth and cattle pressure should
24 influence ¹⁵N signatures. Our results suggest that perennial grasses are more conservative for
25 nitrogen (inhibition of nitrification, less leaching *via* a perennial root system, slower cycling).
26 The increase in leaf $\delta^{15}\text{N}$ with N concentration is steeper in *L. togoensis* than in the three other
27 grasses. No significant difference was found between the ¹⁵N signatures of the four plots. Our

28 results on ^{15}N signatures and the fact that perennial grasses are much more abundant in the
29 plots that are less grazed and have deeper soils, confirm that the switch from perennial to
30 annual grasses is linked to a degradation in soil fertility and pasture quality. This suggests that
31 ^{15}N signatures can be used as indicators of fertility.

32

33 *Keywords:* ^{15}N , nitrogen cycling, savanna, perennial grass, annual grass, herbivore, Burkina
34 Faso

35

36 **1. Introduction**

37 Plants influence nutrient cycling and their long term availability (Chapman et al.,
38 2006; Hobbie, 1992), and this feedbacks on their own growth and primary productivity. This
39 is achieved through many mechanisms (Hobbie, 1992; Knops et al., 2002). This allows them
40 to cope with abiotic constraints of ecosystems such as the lack of mineral nutrients and
41 contrasting soil types and with biotic constraints such as herbivory or competition between
42 plants (Craine, 2009; Grime, 2001). Among all possible strategies, the distinction between
43 perennial and annual plants is particularly relevant in terms of nitrogen cycle and competitive
44 ability (Grime, 1977, 2001). Annuals are viewed as opportunistic. They need to acquire their
45 resources as quickly as possible and benefit from the immediately available mineral resources.
46 Perennials are more likely to influence local nutrient cycles and to benefit from such
47 modifications because their root system, their root exudates and their litter (roots and leaves)
48 interact for a longer period with the same patch of soil and soil micro-organisms living in this
49 patch (Vinton and Burke, 1995). Perennials grow slower than annuals but may benefit from
50 sparing soil mineral nutrient resources, from increasing the local availability of these
51 resources and from decreasing nutrient losses. It has for example been shown in tropical

52 savannas that many perennial bunch grasses are able to inhibit nitrification through the release
53 of particular molecules from their root systems (Lata et al., 2004; Subbarao et al., 2007a;
54 Subbarao et al., 2007b), which increases their own biomass (Boudsocq et al., 2009). As these
55 grasses live for several tens of years, they build dense root systems where the close proximity
56 between dead and live roots is likely to induce a very efficient nutrient recycling and reduce
57 nitrogen losses (Abbadie and Lata, 2006; Abbadie et al., 1992). In this context, our primary
58 goal was to compare, from the point of view of nitrogen cycling, two annual and two
59 perennial bunch grasses growing in the same West African savanna.

60 To meet this goal, we measured the natural abundance in ^{15}N of the biomass of these
61 four grasses and of the soil penetrated by their root system. Indeed, ^{15}N abundance is
62 considered as a good indicator of ecosystem functioning (Dijkstra et al., 2008; Robinson,
63 2001; Staddon, 2004; Templer et al., 2007). First, ^{15}N abundance is used to determine the
64 origin of soil organic matter (Boutton et al., 1998; Danso et al., 1993). Similarly, the ^{15}N of
65 biomass gives hints on the origin of the nitrogen. For example legumes, due to symbiotic
66 nitrogen fixation, tend to have lower concentrations in ^{15}N than other plants and thus to
67 decrease soil ^{15}N concentration through their litter. Second, the isotopic fractionation due to
68 the lower reactivity of ^{15}N relatively to ^{14}N allows tracing nitrogen fluxes in the soil. For
69 example, rapid nitrogen cycling and nitrification are thought to increase ^{15}N abundance
70 (Dijkstra et al., 2008), while tighter nitrogen cycling (e.g. less leaching and denitrification)
71 should lower ^{15}N abundances. The difference between plant and soil ^{15}N abundances gives
72 hints on plant preference for ammonium vs. nitrate (Kahmen et al., 2008). Similarly, the
73 discrepancy between the ^{15}N signatures of the biomass of an African perennial bunch grass
74 and bulk soil (lower ^{15}N in the biomass than in the soil organic matter) (Abbadie et al., 1992)
75 suggested that this grass fosters an efficient nitrogen cycling through the recycling of the
76 nitrogen contained in the roots of its dense and localized root system. Taken together, we

77 predict that our two perennial and two annual grasses should have different ^{15}N signatures,
78 which should in turn impact soil signatures. For the same reasons the universal positive
79 correlation between leaf ^{15}N signature and leaf N concentration (Craine et al., 2009b) should
80 also be different between annual and perennial biomasses.

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

97 Taking into account these interactive impacts of plant species and soil type/herbivores
98 on nitrogen cycling and detecting these interactions using ^{15}N signatures is interesting in two
99 contrasted ways. On the one hand, such complex interactions could strongly increase the
100 variability in isotopic signatures, which could blur potential effects of targeted treatments
101 (here the plant species). Documenting these interactions and their effects on isotopic

102 signatures is thus methodologically useful to help designing and interpreting other
103 experiments or field samplings. On the other hand, isotopic signatures might allow analyzing
104 the impact of herbivores and different soil types on nitrogen cycling and might help
105 disentangling the interactions between herbivores or soil types and plant species.

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

115 **2. Material and methods**

116 *2.1. Study site*

117 The study site is the third management unit of the protected forest of Dindéresso
118 (FCD) in the West of the town of Bobo-Dioulasso, at the altitude of 390 m (11°12.494' north,
119 4°24.159' west). The climate is South-Sudanian: there is a wet season from May to October
120 and a dry season from November to April. The area is located between the 900 and 1250 mm
121 isohyets. 1254 mm of rain fell in 2010 but only 831 mm in 2011. The mean annual
122 temperature is 28°C. The whole forest lies on sedimentary rocks and our own study area lies
123 on Bobo-Dioulasso sandstone. Soils are tropical ferruginous leached soils indurated or not.
124 Vegetation consists in a shrub savanna grazed by cattle. It is characterized by the following

125 dominant shrub/small tree species: *Vitellaria paradoxa*, *Terminalia laxifolia*, *Detarium*
126 *microcarpa*, *Parkia biglobosa*, *Guiera senegalensis*, *Combretum nigricans*, *Gardenia*
127 *ternifolia*. The herbaceous layer is dominated by grasses. The main annual grasses are
128 *Andropogon pseudapricus*, *Loudetia togoensis*, *Microchloa indica*. The main perennial
129 grasses are *Andropogon gayanus*, *Andropogon ascinodis*, *Hyparrhenia subplumosa*,
130 *Schizachyrium sanguineum* and they are all bunch grasses. There are some legumes (*Cassia*
131 *mimosoides*, *Indigofera trichopoda*, *Zornia glochidiata*, *Tephrosia pedicelata*, *Tephrosia*
132 *bracteolata*), Cyperaceae (*Fimbristylis hispidula*) and other forbs (*Waltheria indica*, *Pandiaka*
133 *heudelotii*, *Spermacoce stachydea*, *Striga hermonthica*). Grass aboveground biomass and
134 necromass are burnt each year by bushfires.

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

146 2.2. Soil and biomass sampling

147 The study focusses on four of the dominant grass species: *Andropogon pseudapricus*,
148 *Loudetia togoensis* (annuals), *Andropogon ascinodis* and *Andropogon gayanus* (perennial

149 bunch grasses). The four grasses are cespitosus but the tussocks of the two perennials are
150 much larger than the ones of the two annuals. The four species have C₄ photosynthesis
151 (Breman and De Ridder, 1991). As a control, bare soils were also sampled. In each of the four
152 blocs, 2 or 3 replicates of each plant species or bare soil areas were randomly selected: an
153 individual for perennial bunch grasses, a small patch covered of individuals of the same
154 species for annuals and a small patch deprived of plants for bare soils. In total, 10 replicates
155 were sampled for each species and bare soils and they were spread over the 4 blocs. Taken
156 together, 50 sampling units (plant species or bare soil) were investigated. In each sampling
157 unit a soil sample was collected in June 2011 from the 0-10 cm layer using a core auger (5 cm
158 diameter). This sample was taken in the middle of tussocks for perennial grasses. Soil samples
159 were then air dried in the shade and sieved at 2 mm. Leaves and roots were collected from
160 each annual or perennial grass sampling unit. Roots were collected using a core auger (5 cm
161 diameter) and washed with water. Leaves and roots were oven-dried at 70°C for 72 hours. In
162 total, 40 samples of roots, leaves and soil were analyzed as well as 50 soil samples (40
163 samples for annual and perennial grasses and 10 for bare soils).

164 2.3. Soil and biomass analyses

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

$$170 \quad \delta^{15}\text{N} (\text{‰}) = \left(\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right) 1000,$$

171 The international standard for N is the atmospheric N₂.

172 2.4. Statistics

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

183 3. Results

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

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

196 Biomass $\delta^{15}\text{N}$ is overall higher under the two annual than under the two perennial
197 grasses (Fig .1 and Table 2). Both for roots and leaves there is a significant effect of species
198 and the bloc on biomass $\delta^{15}\text{N}$ but the interaction between these two factors is not significant
199 (Tab. 2). For roots, the $\delta^{15}\text{N}$ is higher (Fig. 2) in the two annuals (respectively 2.60 and 1.26
200 ‰ for *A. pseudapricus* and *L. togoensis*) than in the two perennials (respectively -0.72 and -
201 0.99 ‰ for *A. ascinodis* and *A. gayanus*) and in bloc 1 than in blocs 3 and 4 (respectively
202 1.44, -0.17 and -0.09 ‰ for blocs 1, 3 and 4). This corresponds to an increase in $\delta^{15}\text{N}$ of about
203 2.8 ‰ between perennials and annuals. For leaves, the $\delta^{15}\text{N}$ is higher (Fig. 2) in *A.*
204 *pseudapricus* (2.74) than in the three other species (respectively 0.67, -0.59 and -0.30 ‰ for
205 *L. togoensis*, *A. ascinodis* and *A. gayanus*) and in blocs 1 and 2 than in bloc 4 (respectively
206 1.74, 1.18 and -0.53 ‰ for blocs 1, 3 and 4). Overall, the results on soil and biomass $\delta^{15}\text{N}$
207 support our hypothesis that perennial grasses lead to lower $\delta^{15}\text{N}$ than annuals (first hypothesis
208 in the Introduction). However, these results are at odds with our third and fourth hypothesis.

209 Both for roots and leaves there is a significant effect of species and the bloc on the
210 biomass N concentration but the interaction between these two factors is not significant (Tab.
211 2). Root N concentration is higher (Fig. 2) in the two annuals (respectively 4.09 and 3.65 mg
212 g^{-1} for *A. pseudapricus* and *L. togoensis*) than in the two perennials (respectively 2.52 and
213 3.71 mg g^{-1} for *A. ascinodis* and *A. gayanus*) and in blocs 1, 2, 3 (respectively 3.39, 3.72 and
214 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
215 to an increase in N root concentration of about 48 % between perennials and annuals. Leaf N
216 concentration, as $\delta^{15}\text{N}$, is higher (Fig. 2) in *A. pseudapricus* (10.55 mg g^{-1}) than in the three
217 other species (respectively 6.32, 6.20 and 7.41 mg g^{-1} for *L. togoensis*, *A. ascinodis* and *A.*
218 *gayanus*) and in blocs 2 and 3 than in bloc 4 (respectively 8.24, 8.26 and 6.41 mg g^{-1} for blocs
219 2, 3 and 4).

220 A first ANCOVA shows that there is an overall positive effect of the biomass N
221 concentration on the $\delta^{15}\text{N}$ and that the slopes of the relation between the two variables is
222 steeper for roots than for leaves (Fig. 3). A second ANCOVA shows that for roots, the
223 relation is the same for all species ($\delta^{15}\text{N} = 1.95 N_{\text{R}} - 5.64$, where N_{R} is the root biomass N
224 concentration). A third ANCOVA shows that for leaves the relation varies significantly
225 between species (Fig. 3): the slope is steeper for *L. togoensis* ($\delta^{15}\text{N} = 1.33 N_{\text{L}} - 7.74$, where N_{L}
226 is the leaf biomass N concentration) than for the three other species ($\delta^{15}\text{N} = 0.50 N_{\text{L}} - 3.78$).
227 These results only partially support our second hypothesis (see the Introduction). We also
228 checked that the bloc does not influence the relation between $\delta^{15}\text{N}$ and N concentration in
229 roots and leaves.

230 **4. Discussion**

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

238 *4.1. Impact of grasses on $\delta^{15}\text{N}$*

239 The $\delta^{15}\text{N}$ values are higher in the soil of annual grasses and bare soil than in the soil of
240 perennial grasses and, consistently, the same qualitative pattern is found for the $\delta^{15}\text{N}$ of the
241 grass biomasses. Since the source of nitrogen should be overall the same for plants growing
242 next to each other, this pattern must be due to fine scale differences between species in the

243 various nitrogen fluxes leading to isotopic fractionation. The observed higher soil $\delta^{15}\text{N}$ under
244 annual grasses and in bare soil can be interpreted as an indicator of increased rates in nitrogen
245 cycling (mineralization, nitrification and denitrification) that also foster nitrogen losses
246 (Nacro et al., 2004; Templer et al., 2007; Templer et al., 2008). Indeed, fractionation likely
247 leads to an enrichment of the residual pool in ^{15}N because ^{14}N , the lighter of the two stable
248 isotope, is more reactive (Mariotti et al., 1981). Thus, in the case of increased rates of nitrogen
249 cycling, ^{14}N is more likely to leave the ecosystem through denitrification and leaching, which
250 leads to an increase in soil $\delta^{15}\text{N}$.

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

264 Other studies have shown that soil and biomass $\delta^{15}\text{N}$ values depend on plant species
265 (Kahmen et al., 2008; Kriszan et al., 2009; Nadelhoffer et al., 1996; Templer et al., 2005;
266 Wang et al., 2010). As hypothesized in the introduction, we suggest that perennial grasses are
267 able to influence the nitrogen cycle in a way that increases the availability of nitrogen in their

268 favor. The fact that $\delta^{15}\text{N}$ values of bare soils are identical to the values of annual grasses
269 supports this rationale. Several mechanisms leading to a lower fractionation below perennial
270 bunch grasses than between these grasses and below annuals are probably leading to this
271 general pattern: (1) Many African perennial bunch grasses (e.g. *Hyparrhenia sp.*, *Brachiaria*
272 *sp.*) have been shown to inhibit nitrification (Lata et al., 2004; Subbarao et al., 2007a;
273 Subbarao et al., 2007b). Perennial grasses are able to build a dense and perennial root system
274 so that (2) the proximity between dead and living roots reduces the risk of leaching of the
275 mineral nitrogen released by root decomposition (Abbadie and Lata, 2006; Abbadie et al.,
276 1992) and (3) rates of leaching are likely to be further decreased by the fact that living roots
277 are always present and able to take up available mineral nutrients (Joffre, 1990). Testing fully
278 our hypotheses and interpreting thoroughly the ^{15}N signatures will off course require (1)
279 documenting precisely nitrogen fluxes such as nitrification and denitrification potentials, (2)
280 measuring the ^{15}N signatures of all nitrogen sources, (3) taking into account mechanisms that
281 could complicate the interpretation of ^{15}N signatures such as nitrogen fixation by free bacteria
282 or endophytes (Elbeltagy et al., 2001).

283 4.2. Impact of soil and herbivores on $\delta^{15}\text{N}$

284 Herbivores (Aranibar et al., 2008; Craine et al., 2009a; Frank and Evans, 1997; Frank
285 et al., 2000) and soil properties (Abadín et al., 2010) have often been shown to impact soil
286 $\delta^{15}\text{N}$, which is an indication of modifications of nitrogen cycling. In particular, herbivores
287 impact nitrogen cycling and aboveground-belowground linkages both directly through
288 changes in plant growth and physiology and alterations of the quality and quantity of mineral
289 and organic resources and, indirectly, through changes in the functional composition of
290 vegetation (Bardgett and Wardle, 2003). Here, the species-specific $\delta^{15}\text{N}$ of the soil does not
291 depend on the four blocs while root and leaf $\delta^{15}\text{N}$ tends to be higher in the blocs with the

292 higher cattle pressure (and shallower soil). This suggests (1) that increasing cattle pressure
293 and decreasing soil depth has a relatively weak direct effect on nitrogen cycling and/or that
294 this effect is recent (effects on biomass $\delta^{15}\text{N}$ but no effect on soil $\delta^{15}\text{N}$), (2) that this effect
295 tends to increase the rate of nitrogen cycling and nitrogen losses (Dijkstra et al., 2008;
296 Templer et al., 2007).

297 Besides, our sampling design is based on measurements made at the scale of
298 individual tufts for perennial grasses and small mono-specific patches for annual grasses and
299 we investigated equally the same four grass species in the four blocks. In fact, grazing
300 pressure and soil depth do not change the impact of each grass species on soil $\delta^{15}\text{N}$ but these
301 factors likely strongly impact nitrogen cycling and ^{15}N signatures, indirectly, through their
302 effects on vegetation. Perennial grasses are dominant in the two blocs with deeper soils and
303 lower grazing pressures, while annual grasses are dominant in the two other blocs. This would
304 be the hint that cattle and shallow soils slow down or even reverse the normal succession,
305 which is one of the main mechanisms through which herbivores impact ecosystem
306 functioning (Bardgett and Wardle, 2003). Indeed, annuals normally start to grow in fallows
307 and are progressively replaced by perennials (Bilgo et al., 2006; Somé, 1996). Thus, overall,
308 at the scale of the whole savanna lower soil depth and higher cattle pressure lead to higher
309 $\delta^{15}\text{N}$ values through a switch in the dominant grass species (see the first section of the
310 Discussion). Note that the direct effects of cattle pressure also lead to an increase in $\delta^{15}\text{N}$, at
311 least in biomasses. This is overall a mark of a faster nitrogen cycling and higher nitrogen
312 losses. This is in line with published results (Frank and Evans, 1997; Patra et al., 2006)
313 emphasizing cases of negative effects of herbivores on soil fertility. This also suggests that
314 our blocs 1 and 2 are overgrazed while a milder grazing pressure (blocs 3 and 4) do not lead
315 to such negative effects both in terms of (1) the switch from perennial to annuals grasses and
316 (2) the long-term soil fertility.

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

325 *4.3. Impacts of grasses, soil type and herbivory on nitrogen concentrations*

326 The more efficient nitrogen cycling fostered by perennial than by annual grasses and
327 suggested by $\delta^{15}\text{N}$ values could have led to higher soil nitrogen concentrations for perennials
328 than for annuals and bare soils. Perennial grasses have indeed been found to increase nitrogen
329 content within their root systems (Jackson and Caldwell, 1993; Vinton and Burke, 1995).
330 Such a pattern was not found, and instead, soil nitrogen content depends on a complex
331 interaction between plant species and bloc. This pattern is difficult to explain in details. It
332 seems that the likely interactions between plant species impact on nutrient cycling and grazing
333 by cattle or soil depth is more influential for soil N concentration than for soil or biomass
334 $\delta^{15}\text{N}$. The pattern must be linked to the fact that the likely reduced rate of nitrogen losses
335 under perennials does not necessarily lead to the buildup of the nitrogen stock within the soil
336 because perennial grasses also store a high amount of nitrogen in their root and shoot
337 biomass. Such an apparent discrepancy between fluxes and stocks was observed in Lamto
338 savanna in Ivory Coast where subpopulations of perennial grasses controlling or not
339 nitrification lead to 2-fold differences in grass biomass but not in soil N content (Lata et al.,
340 1999). Our results on soil carbon concentration confirms the idea that in this savanna

341 perennial grasses improve their own access to nitrogen but do not necessarily increase locally
342 soil C or N concentrations.

343 The pattern of nitrogen concentration within grass biomass is simpler: in leaves the
344 nitrogen concentration is higher in *A. pseudapricus* biomass than in the three other species, in
345 roots the nitrogen concentration is higher in the two annuals than in the two perennials. An
346 explanation could be that annual grasses tend to have higher nutrient concentrations than
347 perennials because they favor a quick growth (Craine et al., 2012; Garnier and Vancaeyzeele,
348 1994). This is supported by our root results but only partially by our leaf results: only one
349 annual (*A. pseudapricus*) has a higher leaf N concentration than the two perennials. Nitrogen
350 concentration depends on the availability of mineral nitrogen but is also linked to plant
351 ecophysiological traits such as the capacity to take up nitrogen or nitrogen use efficiency but
352 we so far do not have a clear explanation for the higher value observed for *A. pseudapricus*
353 leaves.

354 4.4. Relation between $\delta^{15}\text{N}$ and N concentration in biomasses

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

364 We expected different slopes for the relation between leaf N concentration and $\delta^{15}\text{N}$
365 between annuals and perennials. Somehow, a species for which N concentration increases
366 quicker with $\delta^{15}\text{N}$ is able to allocate more nitrogen to its leaves for a given rate of nitrogen
367 cycling and a given rate of openness of this cycling (Dijkstra et al., 2008; Templer et al.,
368 2007). This species would be more efficient in terms of exploitation of nitrogen than species
369 with N concentrations increasing slower with $\delta^{15}\text{N}$. We did find a steeper relation between N
370 concentration and $\delta^{15}\text{N}$ for the annual species *L. togoensis*. However, we found the same
371 relation for the other annual and the two perennials. All factors influencing the $\delta^{15}\text{N}$, e.g.
372 mycorrhization and the type of mycorrhiza (Craine et al., 2009b), could explain this
373 difference between *L. togoensis* and the three other species. More information would be
374 needed to interpret this result.

375 Our results on the relation between root N concentration and root $\delta^{15}\text{N}$ are consistent
376 with the comparison of 90 grass species across four regions of the world (Craine et al., 2005).
377 This relation parallels the relation between leaf N concentration and leaf $\delta^{15}\text{N}$ but roots have
378 lower N concentrations than leaves so that the $\delta^{15}\text{N}$ increases more steeply with N
379 concentration for roots than for leaves. For roots the relation between N concentration and
380 $\delta^{15}\text{N}$ is the same for the four species while, for leaves, *L. togoensis* displays a particular
381 relation. This suggests that the particular strategy of this species for N management aims at
382 increasing leaf N concentration and photosynthesis and not at improving the acquisition of
383 soil resources.

384 **5. Conclusion**

385 Our main conclusion remains that perennial grasses decrease soil and biomass ^{15}N
386 signatures relative to annual grasses and bare soil. This confirms the usefulness of ^{15}N as an
387 integrative tool to assess nitrogen cycling (Kahmen et al., 2008; Templer et al., 2007). This

388 suggests that perennials better control nitrogen cycling through a slower cycling and lower
389 rates of nitrogen losses through leaching and denitrification: perennial grasses would have
390 evolved particular mechanisms to improve nitrogen cycling in their favor, which would
391 ultimately feedback on their growth allowing them to accumulate more biomass. These
392 arguments are further supported by the fact that annual grasses can be viewed as *r* or ruderal
393 species in comparison to perennial grasses (Grime, 1977, 2001). In annuals, evolution should
394 have selected strategies that favor the immediate use of mineral resources. Perennials, on the
395 contrary, should benefit from strategies allowing them to make the best use of mineral
396 resources on the long term, which should lead to lower rates of nutrient cycling and lower
397 rates of nutrient losses.

398 The mechanisms evolved by perennials could be involved in the high primary
399 productivity of Guinean humid savannas (Boudsocq et al., 2009; Gignoux et al., 2006) and
400 could increase the primary productivity of Sudanian savannas dominated by perennial grasses
401 (for example, here in Burkina Faso). In turn, our results are consistent with the idea that
402 annual grasses are a mark of soil and vegetation degradation, and that annual grasses tend to
403 replace perennials in less fertile soil conditions and after over-grazing (Belsky and
404 Blumenthal, 1997; Burke et al., 1998; César, 1989; Derner et al., 1997; Rossignol et al.,
405 2006). Here, because the higher cattle pressure occurs in the area of shallower soils it was *a*
406 *priori* not possible to disentangle their possible effects, which should be possible with an
407 experimental approach excluding cattle from some patches. Anyway, our results suggest that
408 soil or biomass ¹⁵N signatures could be used as a diagnostic tool for the degradation of
409 pastures and the fertility of their soils.

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

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	Df	Soil $\delta^{15}\text{N}$	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

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

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	Df	Root $\delta^{15}\text{N}$	Root N	Leaf $\delta^{15}\text{N}$	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

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588 Table 3: Means and standard deviations (in parentheses) for biomass and soil N
589 concentrations (mg g⁻¹) and δ¹⁵N (‰).

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	Soil δ ¹⁵ N	Soil N	Root δ ¹⁵ N	Root N	Leaf δ ¹⁵ N	Leaf N
<i>A. ascinodis</i>	3.98 (0.55)	2.09 (2.76)	-0.72 (0.86)	2.52 (0.31)	-0.59 (1.32)	6.20 (1.95)
<i>A. gayanus</i>	3.88 (0.60)	2.03 (2.21)	-0.99 (0.73)	2.71 (0.47)	-0.30 (0.52)	7.41 (1.17)
<i>A. pseudapricus</i>	4.38 (0.34)	3.01 (2.37)	2.60 (1.78)	4.09 (0.45)	2.74 (2.06)	10.55 (2.33)
<i>L. togoensis</i>	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)	-	-	-	-

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

603

604 Figure 2: Boxplots for soil C concentration. Medians are displayed together with 25th and
605 75th quartiles. Treatments and combinations of treatments that are displayed correspond to
606 significant effects as determined by the ANOVAs (Table 2). Different letters denote
607 significant differences between treatments. When the interaction between bloc and grass
608 species is significant different letters denote differences between blocs within a grass species.
609 1, 2, 3, 4, blocs; *A. pseuda* stands for *A. pseudapricus*.

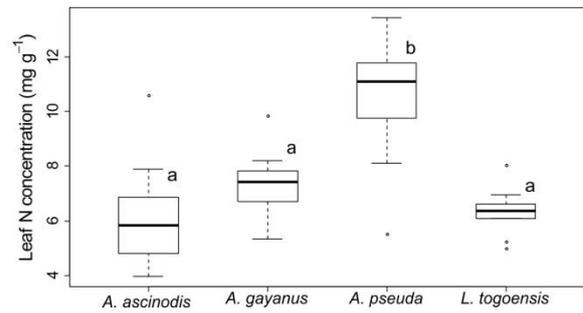
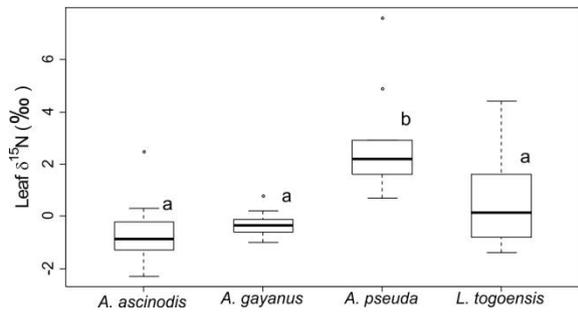
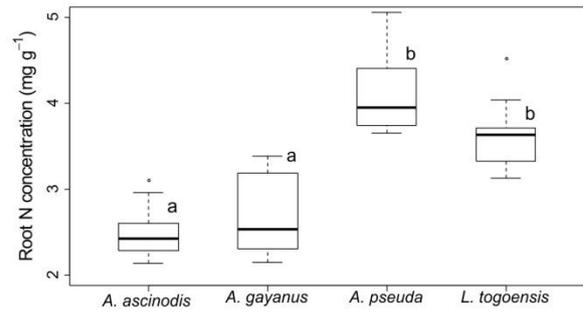
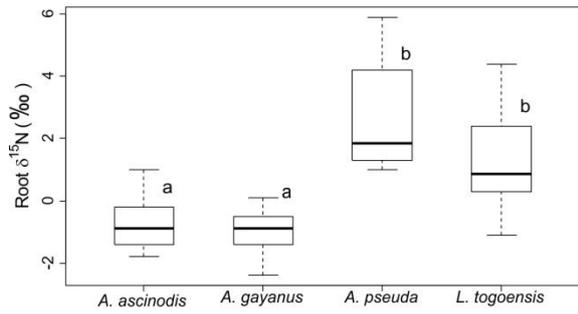
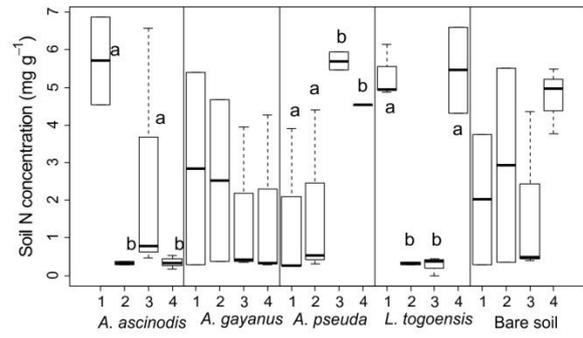
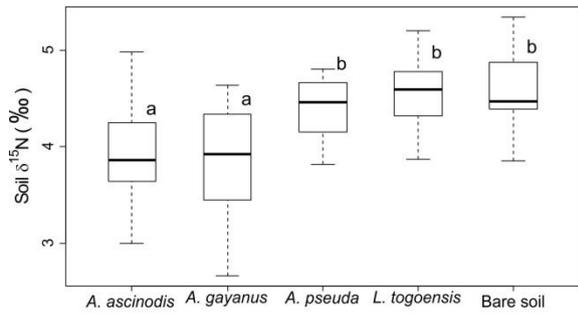
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611 Fig. 3 Regression between biomass $\delta^{15}\text{N}$ and N concentrations. The regression is significantly
612 different between roots and leaves, and within leaves between *L. togoensis* (LT leaves) and
613 the 3 other species (Other leaves).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

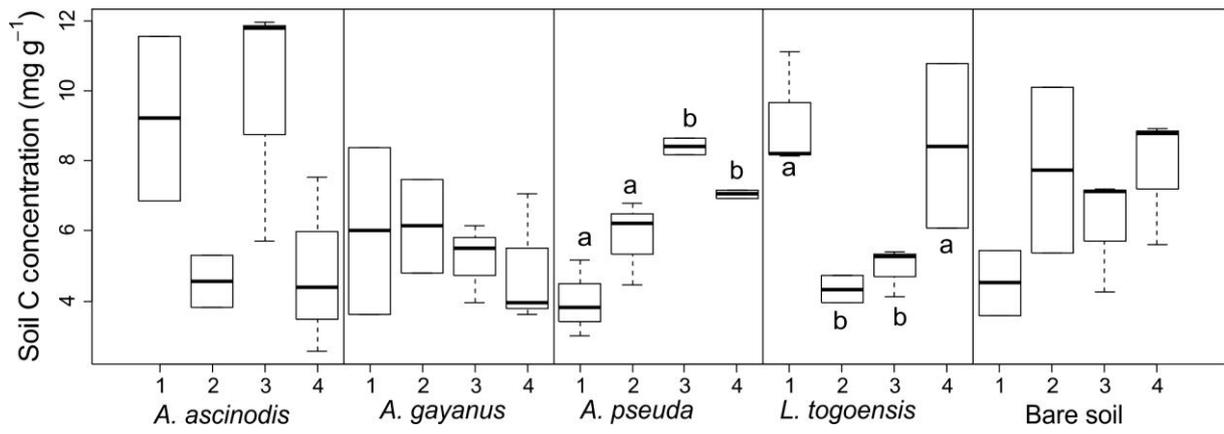
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Fig 1



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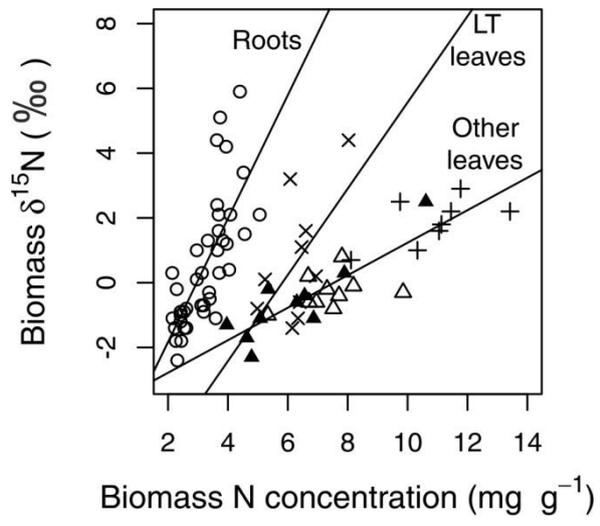
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627 Fig 3



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