The allocation of assimilated carbon to shoot growth in natural grasslands

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1 Introduction

In grasslands, sustained nitrogen loading would increase the proportion of assimilated carbon allocated to shoot growth (A_{shoot}), because it would decrease allocation to roots [4] and also encourage the contribution of fast-growing species with inherently high A_{shoot}. However, *in situ* measurements of carbon allocation are scarce. Hence, it is unclear to what extent species that coexist in grasslands actually differ in their allocation strategy or in their response to nitrogen [5]. In this study, we validated a ^{13}C-labeling approach for estimating A_{shoot} in individuals growing in field stands, and used it to assess A_{shoot} in four grasses coexisting in a natural grassland in Argentina, either fertilized or not with nitrogen, and managed under contrasting defoliation regimes.

2 Materials and Methods

We used a novel steady-state ^{13}C-labeling facility to quantify the daily relative photosynthesis rate (RPR ~tracer assimilated over one light-period) and A_{shoot} (~tracer remaining in shoots after a 100 degree days chase period). The experiments were carried out at the Campo Experimental ‘Colonia Ortiz Basualdo’ of the Estación Experimental Cuenca del Salado (INTA, Argentina, 37°05’S, 57°52’W), at the SE limit of the Río de la Plata grasslands region, in Argentina. Measurements were carried out during winter (Jul.2008) and autumn (May.2009) on four C3 grasses with contrasting morpho-physiological characteristics: *Stipa neesiana*, *S. trichotoma*, *Bromus auleticus*, and *Lolium multiflorum*. Tillers were sampled from plots that were either grazed by cattle or mechanically cut, and either fertilized or not with 10 g N/m^2/y broadcasted as urea.

^{13}C labeling was carried out using the mobile facility described in [3]. Briefly, labeling chambers made of acrylic were placed on top of the vegetation without any attachment to the ground and minimal canopy disturbance. Four labeling chambers were used per treatment – 16 chambers in total. The labeling facility maintained a fairly constant CO_2 partial pressure (370 - 385 µbar) and isotope composition ($\delta^{13}CO_2$ = 398 ‰ = 1.5467 atom%) within the chambers over six to seven hours.

Immediately after labeling, chambers were removed and individuals sampled. Tillers were dug out of the soil and cut at the shoot-root limit. The same sampling procedure was repeated at the end of a 10 days chase period. Tillers sampled outside the chambers served as unlabeled controls for 13C content. Sampled individuals were wrapped in aluminum foil and placed in liquid nitrogen immediately upon harvest, and then stored at -20 °C. Samples were freeze-dried (L-A-B4, Rificor, Buenos Aires, Argentina), weighted, and then milled in a ball mill (MM200, Retsch, Munich, Germany). To quantify the amount of tracer present in non-structural carbon at the end of the chase period, water soluble carbon was extracted.

Carbon and nitrogen content and $^{13}C/^{12}C$ isotope ratio were determined using an elemental analyzer (NA1500, Carlo Erba Strumentazione, Milan) interfaced to a continuous flow isotope mass ratio spectrometer (Deltaplus, Finnigan MAT, Bremen, Germany). The unextracted sample, supernatant and pellet were all analyzed. Samples were measured against a working gas standard previously calibrated against a secondary isotope standard (IAEA-CH6, accuracy ± 0.06 ‰ SD). A laboratory standard (wheat flour) was run after every tenth sample to estimate the precision of the isotope analyses (± 0.09 ‰ SD).

RPR was estimated as the tracer content in tillers harvested just after the labeling pulse times the ratio of total daily incident PAR to PAR incident over the labeling period. A_{shoot} was estimated as the ratio of tracer content in tillers at the end of the chase period to the tracer content in tillers harvested just after the labeling pulse, separating it into its structural ($A_{\text{shoot str}}$) and soluble components ($A_{\text{shoot sol}}$)

3 Results and Discussion

RPR was consistently higher in *B. auleticus* (autumn and winter) and *L. multiflorum* (winter) than in the *Stipa* species (Tab. 1; $P < 0.05$: on average, 1.18 *vs.* 0.86 %/d in autumn, and 2.62 *vs.* 1.20 %/d in winter). In winter, nitrogen fertilizer decreased RPR ($P < 0.05$), and all species showed the same trend. In autumn, nitrogen fertilizer increased RPR ($P < 0.05$), but this was clear only in L. multiflorum (P of interaction species * fertilizer = 0.11).

A_{shoot} varied widely between 0.27 and 0.74, depending on species, nitrogen fertilization treatment and season. Most of the data ranged between approximately 0.30 and 0.60 (Tab. 1). In all cases, little tracer was found in the soluble fraction, 8.8 % on average, and thus $A_{\text{shoot sol}}$ was invariably low (0.01 to 0.08). In consequence, $A_{\text{shoot str}}$ was always close to A_{shoot}. In winter, nitrogen fertilizer increased A_{shoot} in all species, while in autumn it increased A_{shoot} of *B. auleticus* and *S. trichotoma* but no statistically significant difference was detected in *S. neesiana* nor in *L. multiflorum*.

Tab. 1: Relative photosynthesis rate (RPR, % d-1), and proportion of assimilated carbon allocated to shoot growth (A_{shoot}) in four C3 grasses growing in a natural grassland in Argentina, during winter and autumn, in either fertilized (N1) or control plots (N0).

	B. auleticus		*L. multiflorum*		*S.neesiana*		*S.trichotoma*	
	N0	N1	N0	N1	N0	N1	N0	N1
Winter								
RPR	2,14	1,69	4,13	2,51	-	-	1,21	1,19
A_{shoot}	0,32	0,41	0,27	0,37	-	-	0,36	0,60
Autumn								
RPR	1,02	1,34	-	-	0,82	1,08	0,63	0,92
A_{shoot}	0,33	0,56	-	-	0,51	0,41	0,60	0,74

$A_{\text{shoot str}}$ correlated positively with shoot nitrogen concentration, across species, nitrogen fertilizer rates and season (Fig. 1). The association of $A_{\text{shoot str}}$ with nitrogen concentration suggests that apparent seasonal effects in *S. trichotoma* and *B. auleticus* were in fact nitrogen-mediated: plants had higher $A_{\text{shoot str}}$ in autumn than in winter, but also higher shoot nitrogen concentrations.

On the contrary, an intrinsic species effect was evident: *S. trichotoma* had a higher $A_{\text{shoot str}}$ than *B. auleticus* and *L. multiflorum* when compared at similar nitrogen concentrations (Fig. 1). Comparing the regression lines with dummy variables (Type I SS) indicated that these were parallel (test for different slopes: $P = 0.12$) but had different intercepts (test for coincidence of intercepts: $P < 0.01$). A unique regression was therefore fitted: $A_{\text{shoot str}} = 0.11 (\pm 0.06) + 0.22 (\pm 0.03) * \text{species type} + 0.15 (\pm 0.02) * \% N$ ($P < 0.001$, r2 = 0.92, n = 10), where species type equals “0” for *B. auleticus* and *L. multiflorum* and “1” for *S. trichotoma*. *S. neesiana* values were closer to those of *S. trichotoma* in control plots, and to *B. auleticus* and *L. multiflorum* in fertilized plots.

No substantial interspecific difference was observed in the proportion of tracer present in the soluble fraction: it was always relatively low, and similar between *B. auleticus* and *L. multiflorum* (11 % on average) vs. the two *Stipa* species (9 % on average).

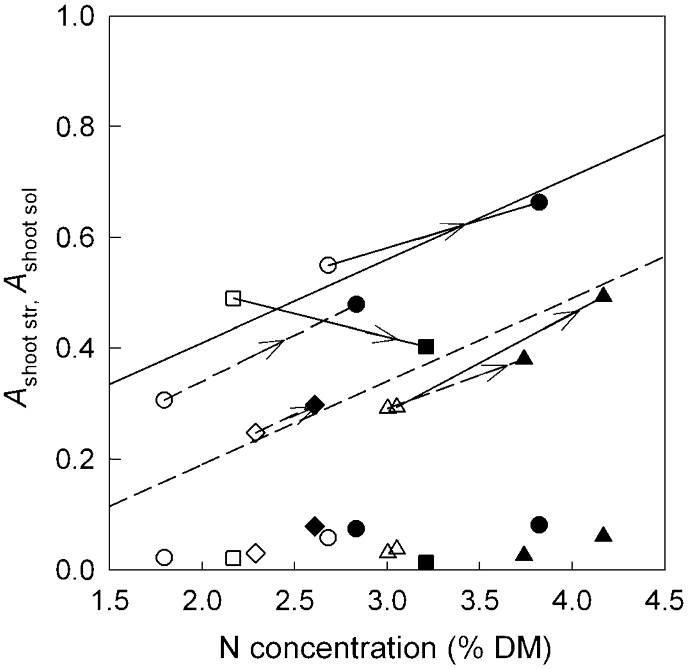

Fig. 1: Relationship between A_{shoot} structural (symbols around regression lines) and A_{shoot} soluble (symbols without regression lines) and nitrogen concentration (as % of DM) for four C3 grasses growing in a natural grassland in Argentina, in winter (dotted lines) and autumn (solid lines), in either control or nitrogen fertilized plots (open and filled symbols, respectively, linked by arrows). The species are: B. auleticus (triangles), L. multiflorum (rhombi), S. trichotoma (circles) and S. neesiana (squares). The regression line is $A_{shoot\ str} = 0.11 (\pm 0.06) + 0.22 (\pm 0.03) * \text{species type} + 0.15 (\pm 0.02) * \% N$ ($P < 0.001$, r2 = 0.92, n = 10), where species type equals “0” for B. auleticus and L. multiflorum (dotted line) and “1” for S. trichotoma (solid line).

A large part of the variability in A_{shoot} was associated with variation in nitrogen status: individuals with a higher nitrogen concentration generally had higher A_{shoot} (Fig. 1). This confirms responses observed in L. arundinaceum [2]. Higher A_{shoot} in response to improved nitrogen nutrition were liekly associated with decreases in allocation of carbon belowground. This agrees with predictions of the functional equilibrium theory and optimal growth models, which postulate that assimilates are partitioned between shoot and root in inverse proportion to their activity, and with empirical results on dry matter allocation in numerous studies (review by [4]). On the contrary, such agreement with theory was less clear in response to shading in a mountain grassland [1].

Species differed in their ability to capture carbon and in their allocation patterns: the two species with higher RPR, *B. auleticus* and *L. multiflorum,* allocated a lesser proportion of the assimilated carbon to the production of shoot tissue than *S. trichotoma* (Tab. 1, Fig. 1). The proportion of assimilated carbon allocated to the soluble fraction was always low. Therefore, the higher A_{shoot} in low RPR-species could not be adscribed to higher allocation to long-term storage.

The interspecific differences in RPR and A_{shoot} found in the present study coincide with the contrasting morphological and physiological characteristics of the *Stipa* species *vs. B. auleticus* and *L. multiflorum*. While the latter are productive species encouraged by nitrogen fertilization, with fast rates of leaf turnover and thus well adapted to frequent defoliation [7], the former have long leaf life spans, slow phyllochrons, and are better adapted to withstand stressful conditions. These results are, to some extent, contrary to the idea that the species that dominate nutrient-rich sites allocate more to shoots. However, we only measured carbon allocation to shoot growth. Allocation to root growth remains to be asessed.

An interesting consequence of the A_{shoot} pattern observed in Fig. 1 is that, opposite to the response expected, the A_{shoot} of grasslands might respond little –even counterintuitively– to sustained nitrogen loading. This is because while fertilization would increase A_{shoot} in all species, it would at the same time encourage the replacement of ‘low RPR-high A_{shoot}’ species by ‘high RPR-low A_{shoot}’ species. Thus, long-term changes in the carbon flux to shoot growth in response to nitrogen would largely reflect changes in total carbon assimilation, rather than in allocation. While this was not the expected response, it explain the results from [6]. These authors compared the A_{shoot} of pastures receiving contrasting phosphorus fertilizer rates over 20 y, and thus with contrasting contributions of species adapted to nutrient-poor vs. nutrient-rich conditions. A_{shoot} was highest (0.49) in low fertility plots, and lowest in the highly fertilized one (0.39). Notably, their data shows, at the community level, the same opposite association between A_{shoot} and daily carbon gain observed in our study.

4 Conclusions

Using steady-state ^{13}C-labeling, the allocation of assimilated carbon to shoot growth was measured *in situ* in individual grass species coexisting in a natural grassland in Argentina. Differences between seasons and nitrogen fertilizer rates correlated positively to variability in shoot nitrogen concentration, in agreement with predictions of the functional equilibrium theory and optimal growth models. The species with higher photosynthesis rates, *B. auleticus* and *L. multiflorum*, allocated substantially less assimilated carbon to shoot growth than *S. trichotoma*. Thus, the two most productive species, which are enhanced the most by fertilization, were able to capture more carbon per unit shoot mass but were less ‘efficient’ at using it to produce shoot tissue. In consequence, nitrogen loading in grasslands may result in little change in carbon allocation to shoot tissue, if increases in A_{shoot} at the species level are offset by decreases associated with the replacement of species.

5 Literatur

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