

1 ***Festuca paniculata* and the grim reaper: carbon and nitrogen storage explain**
2 **dominance in subalpine grasslands**

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23 Running headline : carbon and nitrogen storage in *F. Paniculata* under mowing

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1 **Abstract (234 words)**

2 **Background and Aims:** Cessation of traditional management threatens semi-natural grassland
3 diversity through the colonisation or the increase of competitive species adapted to low
4 nutrient poor conditions. Regular mowing, for example is one of the practices that controls
5 their abundance. This study evaluated the ecophysiological mechanisms limiting short and
6 long-term recovery after mowing for *F. paniculata*, a competitive species which takes over
7 subalpine grasslands from the Central French Alps following cessation of mowing.

8 **Methods:** We quantified the temporal variations in carbon (C) and nitrogen (N) content,
9 starch, fructan and total soluble sugar content in leaves, stem bases and roots of *F. paniculata*
10 during one growth cycle in mown and unmown fields and related them to the dynamics of soil
11 mineral N concentration and soil moisture.

12 **Key Results:** In the short-term, compensatory growth of *F. paniculata* after mowing was N-
13 limited because of N dilution by C increments in the plant organ and due to low soil mineral
14 N and soil moisture. By limiting C storage capacity at the end of the growing season, mowing
15 reduced C allocation to vegetative reproduction during winter and, therefore, limited
16 dramatically the horizontal growth of *F. paniculata* tussocks in the long-term.

17 **Conclusions:** We conclude that mowing reduces the growth of *F. paniculata* tussocks
18 through both C and N limitations in semi-natural subalpine grasslands. Such results will
19 support the understanding of how plant responses to defoliation regulate competitive
20 interactions within plant communities.

21

22 **Key words:** subalpine grassland, mowing, abandonment, diversity, storage

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1 **Introduction**

2 Over the last half-century, land use change in Europe has heavily transformed
3 landscape structure and affected biodiversity. Semi-natural grasslands are closely associated
4 with valued cultural landscapes and high species diversity. These grasslands are now being
5 threatened by rapid changes in agricultural practices: agriculture intensification in some areas
6 and land abandonment in others. This leads to a marked loss in biodiversity and ecological
7 value. Under agricultural abandonment, the reduction of species richness as well as functional
8 diversity can be linked to the promotion of few competitors adapted to nutrient poor soil.
9 These competitors typically include large tussocks such as *Brachypodium pinnatum*,
10 *Deschampsia cespitosa* (Matejkova *et al.*, 2003), *Nardus stricta* (Dorioz and Vanoort 1991),
11 *Molinia caerulea* (Hájková *et al.*, 2009) or *Festuca paniculata* (Quiblier and Senn 2004;
12 Quétier *et al.*, 2007).

13
14 Regular mowing of semi-natural grasslands can limit the abundance of competitive
15 species and maintain high species diversity (Matthias *et al.*, 2001, Rudmann-Maurer *et al.*,
16 2008). Mowing removes a higher proportion of the aboveground biomass of large and tall
17 plants than of small and short plants. This promotes the maintenance of the smaller plants and
18 species richness of the community (Klimeš and Klimešová, 2001). However, this theory,
19 although appealing by its simplicity, does not consider the complexity of whole plant C and N
20 economy. Generally, high stature species have specific organs for C and N storage which
21 allow rapid recovery after cutting. Their response to mowing depends on the proportion of
22 aboveground biomass removed but also on their sensitivity to defoliation, the effectiveness of
23 compensatory growth and the ability to remobilize storage (Chapin *et al.*, 1990). The balance
24 between these processes in the dynamics of acquisition, storage and remobilization of C and

1 N remains unknown. As a consequence, the short-term mechanisms by which mowing
2 decreases the dominance of these high stature species is still undetermined.

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4 Nutrients (N and phosphorous) rather than carbon may also limit dramatically short-
5 term regrowth, especially if the availability of soil nutrients is low. Indeed, since species
6 dominating unmown fields often have conservative nutrient economies (Berendse and
7 Elberse, 1990), their ability to capture new nutrients might be lower than that of typical
8 species of mown grasslands. This is accentuated in cold or dry soils where peaks of N
9 mineralization are very transient (see for example Schmidt *et al.*, 2007). This asynchrony
10 between supply and demand in these ecosystems may limit plant regrowth after mowing.
11 However, while the remobilization of stored carbohydrates has been largely explored under
12 field conditions (*e.g.* Asaeda *et al.*, 2006; Kleijn *et al.*, 2005), most studies investigating N-
13 mobilisation have been conducted with isolated plants grown in controlled condition (*e.g.*
14 Kavanova and Gloser, 2005; Louahlia *et al.*, 1999; Schnyder and de Visser, 1999 but see
15 Kleijn *et al.*, 2005; Louahlia *et al.*, 2000) rather than in field conditions. This limits our
16 understanding of the importance of soil N availability during plant regrowth (Thornton *et al.*,
17 2000).

18

19 In the long term, mowing can influence the dominance of large grasses in the
20 community through the gradual exhaustion of carbohydrate stocks or by reducing C allocation
21 to vegetative reproduction (Wijesinghe and Whigham, 1997). This point is particularly
22 important as clonal species tend to invade communities through growth of large tussocks with
23 inter-connected tillers. However, the mechanisms involved in such longer-term, interannual
24 responses have not been integrated with those documented at the seasonal level for regrowth.

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1 In subalpine semi-natural grasslands of the Central French Alps, *Festuca paniculata*
2 strongly dominates unmown grasslands (Jouglet and Dorée, 1991) in contrast to mown fields.
3 Early spring growth and large carbohydrate storage in stem bases might provide this species
4 with a competitive advantage over other species in the community, along with the release of
5 allelochemicals (polyphenols), which inhibit the growth of the neighbor species (Viard-Cretat
6 *et al.*, 2009). Beside, the large amount of litter produced by this species generally reduces
7 summer soil moisture regimes (Gross *et al.*, 2008) while its low quality restricts soil nitrogen
8 mineralization and thus depletes soils of mineral nutrients (Robson *et al.*, 2007; 2010). Hence,
9 *F. paniculata* expansion causes a rapid change in both plant community composition and
10 ecosystem processes. Regular mowing reduces significantly its abundance, which in turn
11 promotes higher species and functional diversity (Quétier *et al.*, 2007). However, the
12 underlying mechanisms by which mowing alters the growth of *F. paniculata* remain
13 unknown.

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15 In this study, we aimed at evaluating the short and long-term consequences of partial
16 defoliation by mowing on *F. paniculata* at the tiller and at the individual tussock levels, and
17 the underlying mechanisms limiting its recovery. In order to separate seasonal changes from
18 changes caused by defoliation, we examined C and N dynamics in *F. paniculata* throughout
19 its seasonal cycle in mown and unmown fields. We hypothesized that mowing affects in the
20 short-term the regrowth of *F. paniculata* because of limited N content at the time of mowing,
21 and in the long-term limits early spring growth and vegetative reproduction (i.e. low
22 production of new tillers) through reduced C allocation. Temporal variations in C and N
23 content, starch and total soluble sugar content in leaves, stem bases and roots of *F. paniculata*
24 were studied during one growth cycle and were further related to temporal variations in soil
25 mineral N concentrations and soil moisture. Fructans were also quantified because it has been

1 shown that this taxon is generally characterized by high fructan content in its tissues

2 (Chatterton *et al.*, 1990).

3

1 **Material and methods**

2 *Study site and experimental design*

3 The study site is located on the south facing slope of the upper valley of the Romanche
4 River in the central French Alps (45.04°N 6.34°E, 2000 meters above sea level), near the Col
5 du Lautaret (2057 m.a.s.l.). The substrate is homogeneous calc-shale. Mean annual rainfall
6 reaches 956 mm and the mean air monthly temperatures range from -4.6°C in January to
7 11.8°C in July (at Col du Lautaret, 2057 m.a.s.l.).

8 We studied three mown and three unmown semi-natural fields within a uniform grassland
9 area in order to analyse whole plant C and N dynamics of the dominant species, *Festuca*
10 *paniculata* (L.) Schinz and Thellung (Poaceae). *F. paniculata* is a large perennial tussock
11 reaching up to 30-40 cm vegetative height and diameters of up to 14 cm when unmown. Its
12 vegetative growth starts at snow melt, followed by flowering around mid June, while
13 senescence of aboveground parts takes place in October, before snow falls. Its large tussocks
14 are characterized by interconnected basal vegetative shoots (i.e. tillers).

15 Mowing was abandoned in the early 1970's in part of the area due to accessibility to
16 machinery (i.e, unmown fields, Quétier *et al.*, 2007). In contrast, mown fields have remained
17 continuously mown annually for at least six centuries. Mowing occurs late in the season, from
18 early August to early September depending on altitude, due to farmers' constraints to harvest
19 lower areas earlier (i.e, mown fields). Constraining climatic conditions related to altitude and
20 the continental climate allow only one annual harvest due to limited regrowth potential. In the
21 recent decades, these fields have been mown in early August, a practice also consistent with
22 Natura 2000 European network recommendations for the area. In this experiment, mowing
23 was applied to the three mown fields on the 1 Aug. 2008 using a line trimmer and cutting
24 vegetation to about 5 cm height, similar to what is achieved by farmers. Each field was fenced
25 during the growing season to exclude sheep and other possible wild grazers. Details on the

1 corresponding plant communities and soil properties in mown and unmown fields are given in
2 Robson *et al.* (2007; 2010) and Quétier *et al.* (2007).

3

4 *Community composition and F. paniculata abundance*

5 Surveys of botanical composition of these fields have been conducted regularly since
6 2003 at peak vegetation, which occurs around mid July, i.e. before mowing, using the point
7 quadrat method (Quétier *et al.*, 2007). In mown fields the most abundant species are *Meum*
8 *athamanticum* (Apiaceae) and *Festuca nigrescens* (Poaceae). *Festuca paniculata* is by far the
9 most dominant species in unmown fields, followed by two other Poaceae, *Festuca nigrescens*
10 and *Festuca laevigata*.

11 In order to evaluate plant growth strategy, we quantified the density of *F. paniculata*
12 tussocks and its total plant cover visually on the 16 Jul. 2009 in fifteen squares of 50 × 50 cm
13 within each of the three mown and unmown fields.

14

15 *Sampling and sample processing for studying whole plant dynamics of C and N*

16 Sampling started on the 1 May 2008 and finished on the 14 Apr. 2009. Sampling was
17 repeated every 4 weeks, except at the beginning and at the end of the growing season, during
18 which it was repeated every 2 weeks as these are periods when most rapid changes in storage
19 were expected. At each sampling date, three tussocks within the same diameter class were
20 randomly selected in each of the mown and unmown fields. The tussocks were carefully dug
21 out and around 15 to 20 tillers were harvested with their roots. However, it was impossible to
22 sample the whole root system of each tiller. As a consequence, root biomass associated to
23 each tiller was not measured. In addition, we sampled separately the soil under each tussock
24 to estimate soil mineral N concentrations and soil moisture. The samples were rapidly
25 transported to the Alpine Station of Joseph Fourier located at the Lautaret pass (5 minutes

1 away from field sites). An aliquote of soil samples (100g) were immediately frozen at -20°C
2 while another part was used to calculate soil moisture. Tillers were washed with tap water and
3 separated into two groups. The first group was devoted to biometric measurements. Each tiller
4 was dried at 60°C during 48 h and dry-weight (dw) of stem bases and leaves were measured.
5 The second group was devoted to stored compounds analysis. Just after washing, these tillers
6 were frozen at -20°C and then lyophilized (Heto Drywinner, Heto-Holten, Alleröd, Denmark).
7 Before chemical analysis, the lyophilized tillers were separated in three compartments, leaves,
8 stem bases and roots, weighed, pooled by tussock and finally ground to a fine powder using a
9 ball mill (MM301, Retsch GmbH, Germany) for further analysis. Tillers consisted of a top
10 segment of green lamina (hereafter referred to leaves) and a bottom one of cream-coloured
11 sheath (hereafter referred to stem bases). Separation between leaves and stem bases were
12 achieved based on organ function, namely storage vs. photosynthetic function. We thus
13 obtained material for measuring aboveground plant growth, plant C and N dynamics, and soil
14 material for measuring soil mineral N concentrations and soil moisture.

15

16 *Plant chemical analysis*

17 The dynamics of N was analysed by estimating plant total nitrogen content. It was
18 determined using a CHN Elemental Analyser (EA1108, Series 1, Carbo Erba
19 Instrumentazione, Milan, Italy). Dynamics of C was analyzed by measuring contents of
20 starch, Total Soluble Sugars (TSS), fructans and total C. Total C in leaves, stem bases and
21 roots were analyzed by using a CHN analyser (EA1108, Series 1, Carbo Erba
22 Instrumentazione, Milan, Italy). We extracted starch and TSS by the procedure described by
23 Tcherkez *et al.* (2003). Briefly, 50-mg of plant ground powder was suspended with 1-ml of
24 distilled water in an Eppendorf tube (Eppendorf Scientific, Hamburg, Germany). The solution
25 was mixed and centrifuged at 12,000-g for 5-min at 5°C. After centrifugation, the supernatant

1 was used for total soluble sugar quantification, whereas the pellet was stored at -80°C for the
2 starch analyses by HCl solubilization. Total soluble sugars, as sucrose equivalents, were
3 further determined colorimetrically by the anthrone reagent method according to the method
4 proposed by Yemm and Willis (1954). Briefly, the pellet obtained by centrifugation was
5 suspended in 2-ml KOH (1M) and homogenized during 10-min. Then 2-ml of HCL (1M) and
6 0.5-ml of iodine reactive were added. After 15-min in dark, absorbance was measured at 565-
7 nm (Jarvis and Walker, 1993). Starch and total soluble sugar concentration were hence
8 measured for each tussock sampled within each site replicates.

9 The concentration of fructans extracted from the plant was quantified. Briefly, 25-mg
10 of freeze dried plant tissue ground to a fine powder were boiled in 1-mL of 80 % ethanol, and
11 incubated for 15-min at 80°C. After ethanol extraction, the sample was centrifugated at
12 10,000-g for 10-min. The supernatant was preserved and 1-mL of water was added to the
13 pellet. The tube contents were mixed and incubated 15-min at 60°C. After the first aqueous
14 extraction, the sample was centrifugated at 10,000-g for 10-min. The supernatant was
15 preserved and the aqueous extraction was repeated once with the pellet. The three
16 supernatants were pooled, evaporated to dryness under vacuum and the residue was dissolved
17 in 0.5-mL water. Aliquots of carbohydrate extract (100-µL) were passed through
18 minicolumns (Mobicols from MoBITec, Göttingen, Germany) packed, from bottom to top,
19 with 150-µL of Amberlite CG-400 II, formiate-form (Fluka, Buchs, Switzerland), 80-µL of
20 polyvinylpyrrolidone (Sigma-Aldrich, St. Louis, USA), and 250-µL of Dowex 50W X8-
21 400 H⁺-form (Sigma-Aldrich) to remove charged compounds. Fructans were quantified by
22 high-performance liquid chromatography (HPLC) on a cation exchange column (Sugar-Pak
23 300 x 6.5-mm, Millipore Waters, Milford, MA, USA) eluted with 0.1-mM CaEDTA in water
24 using mannitol as the control standard (Guerrand *et al.*, 1996).

25

1 *Soil chemical analysis*

2 Soil moisture was determined gravimetrically by drying the soil at 105 °C during 48h.
3 Soil mineral N was extracted from frozen samples, sieved at 2-mm, with KCl (2 M). The soil
4 extracts were analysed for ammonium (NH₄⁺) and nitrate/nitrite (NO₃⁻/NO₂⁻) concentrations
5 using an FS-IV autoanalyser (OI-Analytical, College Station, TX).

6

7 *Data analysis*

8 Total C and N content of leaves and stem bases were obtained by multiplying the C
9 and the N concentration by the dry weigh of the organs. Total C and N content could not be
10 calculated for the roots. As root N concentration did not vary at the beginning of the growing
11 season, the reliance (R) of early growth on current season soil-derived N was estimated as
12 equaled to:

13
$$R (\%) = 100 ([\Delta N_{\text{mass}_{\text{leaf}}} - \Delta N_{\text{mass}_{\text{stem}}}] / \Delta N_{\text{mass}_{\text{leaf}}})$$

14 With R in % and ΔN_{mass} the variations in N content in both leaves and stem bases between
15 early May and the end of June (mg). We assumed that root biomass during this period did not
16 vary.

17 Plant characteristics and differences between total biomass, TSS, fructan and N content and
18 concentrations in mown and unmown fields were analyzed using the non-parametric
19 Wilcoxon Mann-Whitney tests. Statistical analysis was performed using the average data
20 within each replicate site (for a total of three site replicates in mown and unmown fields
21 respectively). All analyses were performed using the Jmp®8 software (Statistical Analysis
22 System Institute, Inc., Cary, NC).

1 **Results**

2 *Edapho-climatic conditions*

3 Snow melt occurred at the beginning of April in both 2008 and 2009. Soil moisture displayed
4 large variations throughout the growing season and experienced a particularly dry period from
5 the beginning of July 2008 until the end of the growing season (Fig. 1a). Mown fields were
6 significantly drier than unmown fields at the beginning of the growing season (18 May 2008
7 and 3 Jun. 2008) and at the end of the growing season on the 30 Sep. 2008 ($\chi^2_{(1)} = 3.85$,
8 $P=0.04$ in all cases). In both mown and unmown fields, soil NO_3^- concentration were three- to
9 four-fold higher at the beginning of the growing season ($\chi^2_{(1)} = 3.85$, $P=0.04$ between 17 Jul.
10 2008 and 15 Apr. 2009, Fig. 1b) compared to the other periods of the year during which it
11 remained stable (Fig. 1b). This pattern, although less marked for soil NH_4^+ concentration, was
12 also significant ($\chi^2_{(1)} = 3.85$, $P=0.04$ between 17 Jul. 2008 and 15 Apr. 2009, Fig. 1c).
13 Nevertheless soil NH_4^+ decreased more gradually throughout the season than soil NO_3^-
14 concentration. Soil NH_4^+ per g of soil dw was more available than soil NO_3^- (ca. 40 to 50
15 fold) at each date within mown and unmown fields respectively ($\chi^2_{(1)} = 3.85$, $P=0.04$).
16 However, mown or unmown fields did not differ significantly in soil NO_3^- and NH_4^+
17 respectively (Fig. 1b and c, no significant at each sampling date).

18

19 *F. paniculata* abundance and tussock size

20 *F. paniculata* cover (per unit area) reached 14.4 (2.5) % in unmown compared to only 4.0
21 (0.7) % in mown fields ($\chi^2_{(1)} = 3.85$, $P=0.04$). However, tussock density (number of tussocks
22 per square meter) did not differ significantly between mown and unmown fields ($\chi^2_{(1)} = 0.42$,
23 $P=0.41$), with 12.4 (0.2) tussocks per m^2 and 12.5 (2.3) tussocks per m^2 in unmown and mown
24 fields respectively.

25

1 *Seasonal dynamics of growth, carbon and nitrogen storage without mowing*

2 In unmown fields, tillers grew rapidly from May to June 2008 (relative growth rate equaled to
3 $0.69 \text{ mg g}^{-1} \text{ d}^{-1}$, Fig. 2a) and more slowly from mid-June to September 2008 ($0.16 \text{ mg g}^{-1} \text{ d}^{-1}$,
4 Fig. 2a). Biomass was mainly allocated to the leaves (60% of the total aboveground biomass
5 in June – insert in Fig. 2a). However, from July, most of the biomass accumulated in the stem
6 bases (more than 60% in September) (Fig. 2a). Stem bases lose biomass after September and
7 during the winter until spring 2009 (i.e. decrease by a factor of 4.8).

8

9 *C and N accumulate during plant growth*

10 Total Soluble Sugars (TSS) content in leaves and stem bases showed similar temporal
11 fluctuations to total biomass, with a sigmoid increase during the growing season followed by
12 a marked decline during winter (Fig. 3a). The concentrations of TSS remained stable
13 throughout the season in leaves (14.9% in average on a dry weigh basis) as did the
14 concentrations of TSS in roots (9.4% dw in average) (Fig. 3a, insert). By contrast,
15 concentrations of TSS in stem bases increased mostly at the beginning of the growing season
16 and declined progressively from October and during the dormant period in autumn and
17 winter. Hence, high amounts of carbon accumulated in the stem bases during the season
18 (43.6% dw on average) and decreased during autumn and winter.

19 Fructan concentrations showed similar temporal patterns as TSS contents and concentrations
20 in leaves, stem bases and roots (Fig. 3b). Mean fructan concentrations in leaves and roots
21 remained stable throughout the year at 5.9% and 4.2% dw respectively (Fig. 3b, insert).
22 Concentrations in stem bases equaled in average 33.5% dw (Fig. 3b, insert). Surprisingly,
23 fructan concentration in stem bases dropped considerably from the beginning of September,
24 i.e. earlier than TSS (Fig. 3b, insert). No starch was detected in any organ (data not shown).

1 Total N content in leaves and stem bases showed different dynamics (Fig. 3c). In leaves, N
2 content increased sharply and rapidly at the beginning of the growing season, followed by a
3 reduction from the end of July. During spring, N accumulated into leaves but was diluted
4 because leaves also grew. Hence, leaves N concentrations decreased throughout the season
5 (Fig. 3c, insert). In stem bases, N content showed first a decrease until July followed by an
6 increase. Concentration of N in stem bases declined considerably (eight-fold) from the
7 beginning of the growing season while total stem base biomass only increased by a factor 2.5.
8 This indicates N reallocation from the stem bases to the leaves. In parallel, leaf N
9 concentration decreased by 2.6 during the same period whereas leaf total biomass rose by 7.5.
10 This demonstrates that leaf growth rate probably exceeded N supply by the stem bases and
11 roots. Hence, during the first part of growing season, N was provided by the stem bases to the
12 leaves and completed by soil N that was allocated mostly to the leaves (more than 80% of N
13 content were located in the leaves at peak standing biomass). At the same time, root N
14 concentration remained stable throughout the year. From the mid-season, soil N accumulated
15 in the stem bases so that their N concentration increased gradually (Fig. 3c, insert). During
16 winter, stem base N concentration increased strongly from 1.00 % to 1.80 % on a dry weight
17 basis. These results indicate, first, N remobilization from leaves to stem bases at the end of
18 the growing season and, secondly, the effect of decreasing total biomass of stem bases in
19 winter.

20

21 *Seasonal dynamics of growth, carbon and nitrogen storage under mowing*

22 In mown fields, the temporal dynamics of leaf and stem base biomass was similar to that
23 observed in unmown fields (Fig. 2b). Tillers grew rapidly from May to June 2008 (relative
24 growth rate equaled to $1.53 \text{ mg g}^{-1} \text{ d}^{-1}$, Fig. 2b) and more slowly from mid-June to September
25 2008 ($0.07 \text{ mg g}^{-1} \text{ d}^{-1}$, Fig. 2b). Nevertheless, *F. paniculata* accumulated significantly less

1 biomass in the leaves in mown compared to unmown fields (1.7-fold lower at peak standing
2 biomass on the 17/07/08; $\chi^2_{(1)} = 3.85$, $P=0.04$). The decrease during winter was less
3 pronounced and plants started the new season with approximately the same biomass in mown
4 and unmown fields ($\chi^2_{(1)} = 0.43$, $P=0.51$ and $\chi^2_{(1)} = 0.04$, $P=0.83$ for leaves and stem bases
5 respectively on the 14/04/09). In general, mown plants accumulated less biomass during the
6 season.

7

8 *Was the dynamics of C and N modified by mowing?*

9 TSS and fructan content in stem bases differed marginally significantly at $P<0.10$ between
10 mown and unmown fields just after mowing (for example $\chi^2_{(1)} = 3.0$, $P=0.08$ and $\chi^2_{(1)} = 3.85$,
11 $P=0.04$ in leaves and stem bases respectively on the 14 Aug. 2009, Fig. 3a, d and b, e). Thus,
12 less TSS and fructan content accumulated in leaves and stem bases under mowing. This is
13 consistent with the lower biomass of these organs compared to unmown conditions. In
14 contrast, TSS and fructan concentrations were similar in leaves and stem bases compared to
15 unmown fields ($\chi^2_{(1)} = 1.24$, $P=0.25$ and $\chi^2_{(1)} = 0.42$, $P=0.51$ in leaves and stem bases
16 respectively on the 14 Aug. 2009, Fig. 3a, d and b, e, inserts). In roots, TSS and fructan
17 concentrations were higher in unmown vs. mown fields ($\chi^2_{(1)} = 3.85$, $P=0.04$, Fig. 3a, d and b,
18 e, inserts).

19 During winter, TSS consumption from stem bases was slower compared to unmown fields, in
20 absolute and in relative terms as stem bases lost 30% of TSS content compared to 50% in
21 unmown fields. Hence, mowing resulted in a shortage in TSS at the end of the season, which
22 put a strain on reserve accumulation in stem bases for winter consumption.

23 N concentrations were the same in mown and unmown fields for all organs at the beginning
24 of the growing season. N increased while soil N was available (see Fig. 1b, c), but this was
25 followed by only little accumulation of N in the stem bases from July 2008, contrary to the

1 unmown fields (Fig. 3c, f). At peak growing season, total accumulated N content was lower in
2 mown than in unmown fields ($\chi^2_{(1)} = 3.85$, $P=0.04$ in leaves and stem bases on the 17 Jul.
3 2008, Fig. 3c, f). After mowing, stem base N storage decreased. As stem base biomass was
4 also highly affected by mowing, stem bases were then more concentrated in N. This pattern
5 was also particularly marked in leaves: after mowing, leaf N concentration was two-fold
6 greater as compared to unmown fields ($\chi^2_{(1)} = 3.97$, $P=0.04$, Fig. 3c, f, inserts). During winter,
7 stem base N content remained constant (Fig. 3f). As biomass decreased, N concentration in
8 stem bases slightly increased (1.4-fold) whereas root N concentration remained constant
9 throughout the year (Fig. 3f).

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1 **Discussion**

2 ***F. paniculata* growth and C/N storage dynamics during the growing season**

3 *F. paniculata* has characteristic cycles of phenological development, storage and
4 depletion of carbohydrates and growth response to its environment. This species showed the
5 classical V-shaped C reserves cycle, with winter and spring C depletion followed by a period
6 of replenishment completed by mi-July (Menke and Trlica, 1981). Although starch is
7 considered as the major storage compound for cereals and grasses (grains filled by starch) it is
8 completely absent in *F. paniculata* tissues. In contrast, leaves, stem bases and roots had very
9 high total soluble sugar concentrations including large amounts of fructan (Fig. 3b, e). The
10 advantage of fructan as a storage carbohydrate is commonly thought to reside in the
11 utilization of the vacuole as a storage compartment, which would allow plants to exploit
12 constraining environments (Pollock and Cairns, 1991). In these environments, periods of
13 positive carbon balance are short and net mobilization of reserves is therefore required to
14 sustain growth (Pollock and Cairns, 1991). Hence, by maintaining supplies of fructose and
15 sucrose, species obviate the need for transport of carbohydrate over distance as in starch
16 storing species (Bloom *et al.*, 1985; Hendry, 1987). Furthermore, fructan content and its
17 metabolism have been shown to be closely related to freezing tolerance (see Valluru and Van
18 den Ende, 2008 for a review). The presence of C stocks under fructan form in the stem bases
19 and leaf vacuoles might offset possible damages effects associated to frost events (Bloom *et*
20 *al.*, 1985) in addition to providing readily accessible compounds to plant metabolism.
21 Surprisingly, fructan content in stem bases increased strongly during the growing season but
22 decreased from mid-August in contrast to total soluble sugars which still increased until the
23 end of the growing season (see Fig. 3). These results suggest that lower temperature and/or
24 reduced photoperiod at the end of the growing season induce a net depolymerization of

1 fructans with no net loss of carbohydrates as it has been already demonstrated in tubers of *H.*
2 *tuberosus* (Pollock and Cairns, 1991).

3 The analyses of plant growth data revealed that since the beginning of May 2008,
4 plant growth rates increased in both mown and unmown fields (Fig. 2). Leaf emergence and
5 such high relative growth rate could be explained by larger photosynthetic activity and soil N
6 uptake or/and the remobilization of stored C and N. Our results suggest that when relative
7 growth rate is at its maximum, the reliance of mineral N soil increases rapidly. Indeed, under
8 the simplifying assumption of constant root biomass, our calculations showed that, between
9 early-May and the end of June, approximately 67% of the total leaf N content was met by soil
10 N uptake. Hence, while the growth of *F. paniculata* may rely on N stores at the beginning of
11 the growing season, it must rapidly switch to current season soil derived N, as does *Veratrum*
12 *album*, another subalpine species (Kleijn *et al.*, 2005). Similarly, the parallel increases in stem
13 base and total biomass suggests that during this period storage remobilization was not the
14 main factor explaining the increase in dry biomass. Enhancement in photosynthetically-
15 derived carbohydrates, like total soluble sugars (TSS) and total fructans (Fig. 3), likely
16 indicates that dry biomass increase during May-July was caused by increased photosynthetic
17 activity.

18 These results demonstrate that *F. paniculata*, as many others subalpine and alpine
19 species, stores a very large quantity of N and carbohydrate that are not used for growth at the
20 beginning of the growing season (Körner, 1999). Rather, in harsh and constraining
21 environment such as subalpine grasslands, these stores may provide plants with (1) the
22 support for vegetative regrowth (Menke and Trlica, 1981) (2) the ability to bridge temporal
23 gaps between resource availability and resource demand (Chapin *et al.*, 1990), (3) the support
24 to sexual or vegetative reproduction in the absence of photosynthesis (Wijesinghe and

1 Whigham, 1997) and (4) the ability to survive stresses such as defoliation, shading or frost
2 (Kleijn *et al.*, 2005).

3 ***Winter dynamics of C reserves and clonal growth***

4 The decline in total biomass, total soluble sugars and fructan content in stem bases was
5 particularly large during winter. Mild temperature under the snow and thus associated high
6 respiration rates could be partly responsible for this reduction (Wyka, 1999). However *F.*
7 *paniculata* plants located in mown fields did not experience such a decrease during winter
8 demonstrating that the amount of carbohydrates present in storage organs of unmown *F.*
9 *paniculata* at the onset of winter was in excess of that required for survival through
10 dormancy. Thus, it is likely that a large proportion of C and N stocks might be allocated to the
11 production of new tillers. These results are supported by the findings of Viard-Cr tat
12 (unpublished results, see appendix 1) which demonstrated the production of new tillers during
13 winter as for other species from the same *genus* (e.g. *F. arundinacea* in late winter, Garwood,
14 1969; Lafarge and Loiseau, 2002). Hence, we hypothesize that the vegetative reproductive
15 activity of *F. paniculata* may coincide with the accumulation of storage carbohydrates.

16

17 ***Effect of mowing on C and N storage in F. paniculata at individual tiller level***

18 Diverse physiological responses in plant species have been identified in order to compensate
19 tissue removal. They include the reallocation of stocks to remnant organs (Liu *et al.*, 2007),
20 higher photosynthetic C allocation to aboveground biomass (Briske *et al.*, 1996; Zhao *et al.*,
21 2008) or increase in net photosynthetic rate of remnant leaves (e.g. Parsons *et al.*, 1983;
22 Anten and Ackerly, 2001; Zhao *et al.*, 2008). Nevertheless, in the case of *F. paniculata*, we
23 rather observed an under-compensatory growth response especially at the end of the growing
24 season (from mid-August to the end of September). By the 15th of August, total soluble sugars
25 and fructan concentrations and masses decreased in *F. paniculata* stem bases (Fig. 3d, e).

1 Total N concentration in leaves, although increasing after mowing, was greatly reduced
2 compared to early season values due to a dilution of N by C increments (Fig. 3f). Hence, low
3 leaf N probably limited photosynthetic capacity and therefore re-growth. Moreover, as it has
4 been demonstrated in other grasses, once leaves have been cut, mineral N uptake may be
5 dramatically reduced and C supply to roots drastically limited (Kim *et al.*, 1993). Hence, in
6 this situation, the cutting of shoots might trigger a temporary interruption in plant growth
7 (Boucaud and Bigot, 1989; Ta *et al.*, 1990).

8 Other mechanisms may contribute to reduce the compensatory ability of *F. paniculata* to
9 defoliation damage. The period during which defoliation occurs and the available time for
10 recovery can strongly impact the outcome of defoliation (Monson *et al.*, 2006). Grasses
11 withstand greater defoliation during early and rapid growth stages than they do later in the
12 growing season, after most growing is complete (Briske *et al.*, 1996). In the case of *F.*
13 *paniculata*, mowing occurs after peak standing biomass and at a time when the photoperiod is
14 markedly reduced. Beside, soil moisture and nitrogen soil availability were particularly low
15 during this period (Fig.1) and might also limit considerably the re-growth of *F. paniculata*.

16

17 ***Effect of mowing on long-term performance of F. paniculata tussocks***

18 Clonal species can develop compensatory growth in response to cutting by
19 maintaining the number of photosynthetic tillers or by the activation of additional meristems
20 due to the release of apical dominance (McNaughton, 1983). Nevertheless, although *F.*
21 *paniculata* tussock density remained similar in both mown and unmown fields, the total area
22 of each *F. paniculata* tussock, and so the number of tillers per tussock, were strongly reduced
23 in mown compared to unmown fields. This was probably due to a reduced winter production
24 of new tillers although not statistically significant (Viard-Crétat, unpublished results, see
25 appendix 1). Low stored carbohydrate reserves might be perceived as a signal of unfavorable

1 carbon status such that plants restrict reproductive effort (Wyka, 1999). Hence, this species
2 may tend to invest more energy in the re-growth of the cut tillers than in the production of
3 new tillers.

4 Though not explicitly examined in this study, it is likely that by limiting individual
5 growth in the long-term, defoliation also restricts significantly root biomass and associated
6 hyphal networks (Guitian and Bardgett, 2000; Esmaeili *et al.*, 2009) therefore decreasing
7 plant N uptake per unit of plant dry weight in mown fields (Robson *et al.*, 2010). Such
8 mechanisms might explain (1) why at the beginning of the growing season *F. paniculata*
9 displays lower N concentration in both leaves and stem bases in mown fields and
10 consequently (2) why its growth is reduced, especially from June to the end of the growing
11 season. Nevertheless the significance of these mechanisms requires further exploration.

12

13 **Conclusion**

14 The monitoring of growth, carbon and nitrogen content in leaves, stem bases and roots
15 of *F. paniculata* throughout a full annual cycle suggests that, consistent with our hypothesis,
16 compensatory growth of *F. paniculata* following mowing was N-limited, firstly, because of N
17 dilution by C increments in the plant organ and, secondly, due to low soil mineral N
18 concentration and soil moisture. By limiting C storage at the end of the growing season,
19 mowing also reduced C allocation to vegetative reproduction during winter and, therefore,
20 had the potential to dramatically limit the horizontal growth of *F. paniculata* tussocks in the
21 long-term.

22 These results also suggest that, consistent with agronomic trials, mowing or grazing
23 earlier in the season (e.g. mid July) might be more efficient to control *F. paniculata* as total C
24 allocated to leaves reached its highest level during this period. This would induce greater C
25 loss and therefore a stronger reduction of horizontal growth than through current late mowing.

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8 **Figure legends**

9 **Figure 1** Soil moisture (a), nitrate concentration (NO_3^-) (b) and ammonium concentration
10 (NH_4^+) (c) in soils sampled under *F. paniculata* individuals from May 2008 until Apr. 2009 in
11 unmown (white) and mown fields (black). See text for statistical differences between mown
12 and unmown fields. Mean (SE), n=3. NA: data is not available. Arrows indicate mowing date.

13

14 **Figure 2** Stacked graphs of leaf (light grey) and stem base (dark grey) biomass (per tiller) of
15 *F. paniculata* in unmown (a) and mown (b) fields from May 2008 until April 2009. Graphic
16 inserts represent the proportion of total biomass allocated to leaves vs. stem bases throughout
17 the growing season 2008. Mean (SE), n=3. Arrows indicate mowing date and dashed line the
18 end of the growing season. See text for statistical details.

19

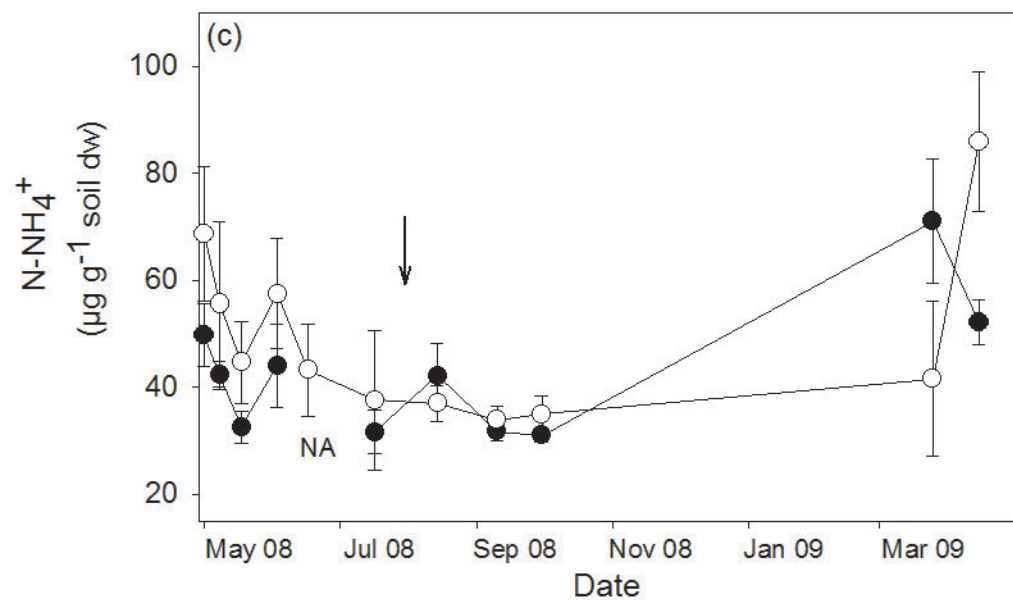
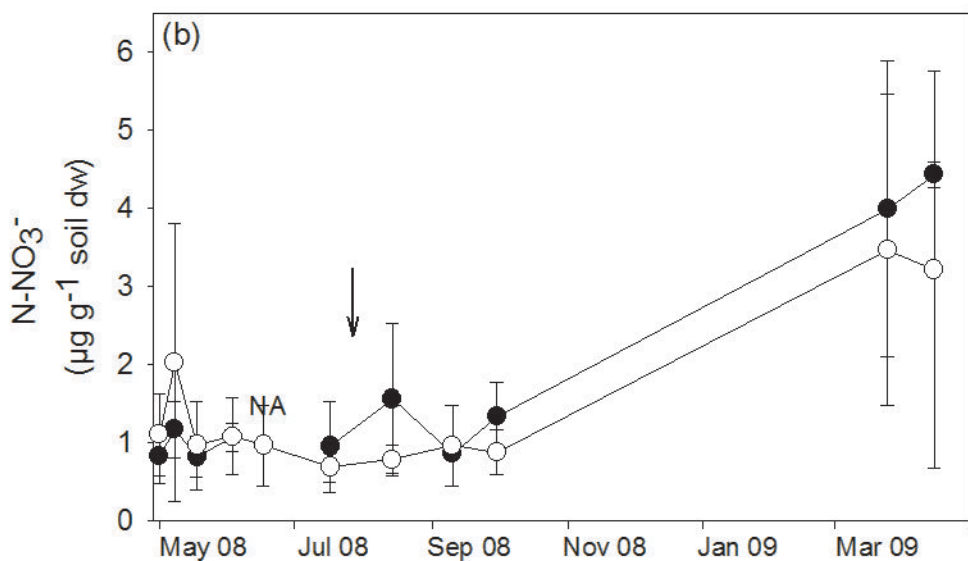
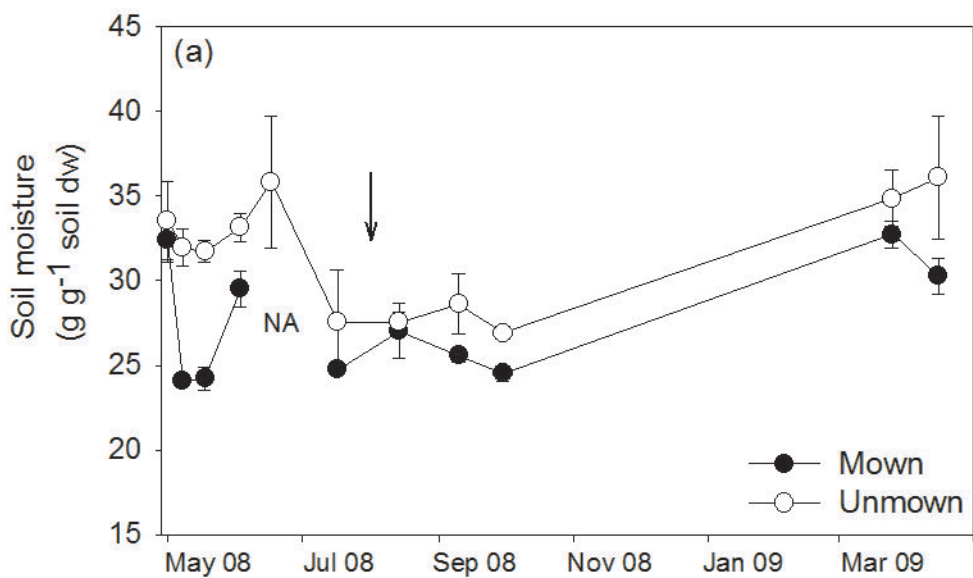
20 **Figure 3** Stacked graphs of total soluble sugar content (a, d), fructan content (b, e) and total N
21 content (c, f) in leaves and stem bases (per tiller) of *F. paniculata* in mown and unmown
22 fields from May 2008 until Apr. 2009. Graphic inserts represent the concentration of fructan,
23 TSS and N (on a dry weigh basis) in the leaves (circle), the stem bases (square) and the roots
24 (triangle) from May 2008 until Apr. 2009. NA: data is not available. Mean (SE), n=3. Arrows
25 indicate mowing date and dashed line the end of the growing season. See text for statistical
26 details.

27

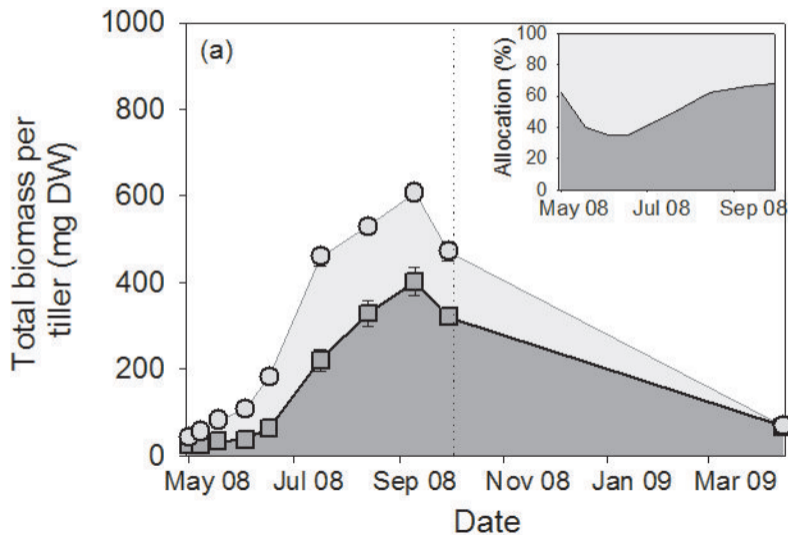
28 **S1** Vegetative growth of *Festuca paniculata* during winter in mown and unmown meadows,
29 measured in production of new ramets in small squares of 10 x 10 cm.

30

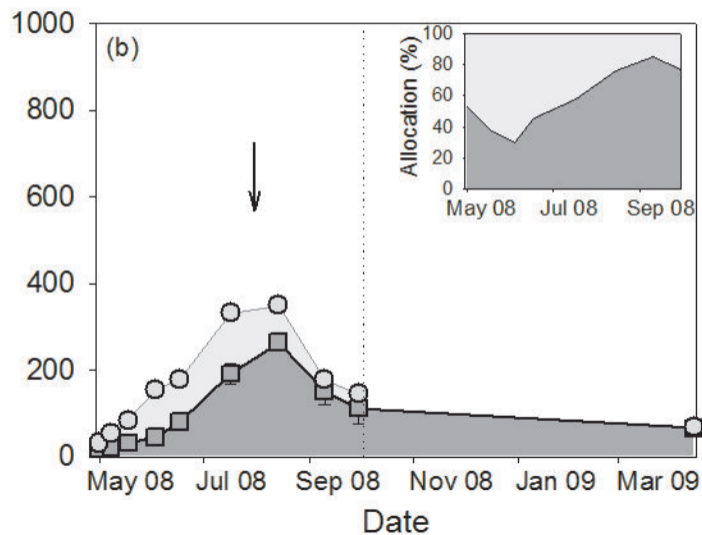
31



Unmown fields



Mown fields



Unmown fields

Mown fields

