Plant clipping decelerates the mineralization of recalcitrant soil organic matter under multiple grassland species

Tanvir Shahzad, Claire Chenu, Cedric Repinçay, Christian Mougin, Jean-Luc Ollier, Sébastien Fontaine

Plant clipping decelerates mineralization of recalcitrant soil organic matter by reducing plant N uptake

Tanvir Shahzad [a*], Claire Chenu [b], Cédric Répinçay [c], Christian Mougin [c], Jean-Luc Ollier [a], and Sébastien FONTAINE [a]

[a] INRA, UR 874 Unité Recherche d'Ecosystème prairial, 234-Avenue du Brézet 63100 Clermont Ferrand, France.

[b] AgroParisTech, UMR BIOEMCO (Biogéochimie et Ecologie des Milieux Continentaux) AgroParisTech Grignon, 78850 Thiverval Grignon, France

[c] INRA UR 251 Physicochimie et Ecotoxicologie des SolS d'Agrosystèmes Contaminés, RD 10- 78026 Versailles Cedex

Summary

Grazing or mowing is central to the management of grasslands and it may alter mineralization of soil organic matter (SOM) and soil C stocks. It has been shown that grazing reduces total soil respiration suggesting decrease in SOM mineralization. However, based on increased soluble C and mineral N in soil after clipping, it has also been suggested that grazing may increase SOM mineralization. Therefore, there is lack of a direct evidence of the effect of grazing on SOM mineralization and mechanism behind it. We examined the effect of clipping on soil-derived CO_2 efflux (*Rs*, SOM mineralization) for six gramineae and one leguminous species typical of temperate grasslands. Continuous ^{13}C labelling of monocultures coupled with a new method of directly measuring *Rs* in herbaceous plants was used. For a model species, *Lolium perenne*, clipping effect on shoot biomass production, mineral N and soluble C in soil, microbial biomass and community composition were also quantified. We show that

clipping decreased the SOM mineralization (*RPE*) within 48 hours and this was common to the seven studied species. For *Lolium perenne*, we show that this reduced SOM mineralization persisted for one month. Moreover, clipping increased mineral N, soluble C and a gram positive bacterial biomarker and decreased a saprophytic fungal biomarker. These results indicate that increased availability of soluble C under clipped plants does not necessarily increase SOM mineralization until microbes that are considered key players in SOM mineralization (i.e. fungi) are not stimulated. They suggest that lower N uptake by clipped plants, as shown by lower N export *via* shoot biomass and increased soil mineral N, favours bacteria over fungi thus decreasing SOM mineralization. Finally, the synchronization between the plant uptake of N and the mineralization of SOM suggested by this study supports the idea that soils under permanent plant cover function as a bank of nutrients for plants favouring primary production and soil carbon storage.

Keywords:

Priming effect, grazing, soil respiration, ^{13}C labelling, PLFA, temperate grassland, microbial community composition

1. Introduction:

Grassland ecosystems contain almost 12% of earth's soil organic matter (SOM) (Schlesinger 1977), 90% of which is estimated to be concentrated belowground in form of roots and SOM (Parton et al., 1993). They are considered potential carbon (C) sinks in the context of increasing CO_2 levels in atmosphere provided they are properly managed (Lal et al., 2007; Li et al., 2008). Managed grasslands cover about 20% of the global terrestrial ice-free surface. Grazing or mowing is central to their management and has been reported to modify below ground soil processes like plant-litter decomposition (Bardgett et al., 1998; Reeder and Schuman 2002; Stark and Kytoviita 2006; Klumpp et al., 2009), nutrient cycling (Bardgett et al., 1998; Mikola et al., 2002) and biodiversity of plants (Collins et al., 1998; Ward et al., 2007) and soil decomposers (Bardgett et al., 2001).

There is contradiction on how the grazing (or any practice analogous to it like clipping, mowing or defoliation) modifies the soil C stocks and fluxes. For example, it has been reported that mowing reduces total soil CO_2 efflux (CO_2 as a result of root respiration and mineralization of plant litter and recalcitrant SOM) by 20-50% despite the increase in soil temperature (Bremer et al., 1998; Wan and Luo 2003; Bahn et al., 2006). It has been suggested that this decrease is due to decreased canopy photosynthesis and reduced C supply from aboveground plant parts to roots, mycorrhizae and rhizosphere microorganisms (Bremer et al., 1998; Craine et al., 1999; Bahn et al., 2006). On the other hand, it has been shown that mowing/defoliation results in increased soluble C, microbial biomass and nitrogen availability in soil (Hamilton and Frank 2001; Henry et al., 2008) although this increase in microbial biomass is short-term and not systematically observed in all studies (Hamilton and Frank 2001; Henry et al., 2008; Bazot *et al*., 2005). Based on their results, Hamilton and Frank (2001) suggested that grazing triggers plant exudation stimulating microbial mineralization of SOM thus liberating mineral nitrogen. Although grazing has been shown to accelerate the

mineralization of plant litter (Klumpp et al., 2009), increased microbial biomass and availability of soluble C does not necessarily imply that there would be increased mineralization of recalcitrant SOM as well because different microbial groups are specialized in mineralizing different types of organic substrates. The acceleration in mineralization of recalcitrant SOM could only happen if those microbes that specialize in it are stimulated in the event of increased availability of labile C (Fontaine et al., 2003). Thus, the effect of grazing on mineralization of recalcitrant SOM remains uncertain, despite the large quantity of C stored in this soil pool, and should be quantified through a direct approach.

The supply of fresh energy-rich C to soil (e.g. under living roots) stimulates SOM mineralization and soil-derived CO_2 efflux (*Rs*) (Broadbent 1947; Bingeman et al., 1953; Wu et al., 1993; Cheng et al., 2003)- a process called rhizosphere priming effect (*RPE*). As a result, fresh C supply by plants and *RPE* are generally positively linked (Dijkstra and Cheng 2007). For example, increased rates of fresh C by plants exposed to elevated CO_2 level in atmosphere has been shown to accelerate SOM mineralization as compared to plants at ambient CO_2 levels (Phillips et al., 2010; Drake et al., 2011). In order to quantify *RPE* induced by plants, current methods are based on a physical separation of soil and plant compartments during the measurement of respiration fluxes. This separation allows the measurement of CO_2 released from soil compartment and avoids any plant uptake of soil-originated CO_2 through photosynthesis. As consequences of this separation, current method is only applicable to single stem plant and trees and *RPE* induced by herbaceous plants has not yet been quantified. Developing a new method for quantifying the *RPE* under herbaceous plants is indispensable to determine the effect of grazing on recalcitrant SOM mineralization.

In addition to microbial biomass, grazing has been shown to cause changes in microbial community composition. For example, an increasing intensity of grazing has been shown to decrease fungi: bacteria ratio (Bardgett et al., 2001; Klumpp et al., 2009). This

microbial shift can result in important biogeochemical changes because soil microbial groups have distinct preferences in term of organic matter sources (van der Heijden et al., 2008; De Deyn et al., 2008). For example, the decrease in abundance of fungi relative to bacteria due to an intensified grazing has been postulated to cause increased rate of nutrient cycling and decreased retention of C and N in the litter pool (Bardgett et al., 2005). However, this decrease in fungi abundance could decelerate SOM mineralization since fungi have been suggested as actors of the priming effect in soil incubation studies (Fontaine et al., 2011). Linking the change in SOM mineralization in response to grazing with microbial community composition could verify the key role of fungi in SOM mineralization in a real plant-soil system.

Here we use *Lolium perenne* as a model species to test if plant clipping causes any change in soil-derived CO_2 efflux (i.e. SOM mineralization) at short (within 48 hours) and long term (after one month). Continuous ^{13}C labelling of plants was used to distinguish soil-derived (*Rs) and* plant-derived respiration (*Rp).* Rhizosphere priming effect was calculated as the difference between *Rs* from planted soils and *Rs* from control bare soil. As separation of soil and plant compartments is not possible for herbaceous plants, we developed a new method for quantifying *RPE* that avoids any plant uptake of soil-originated CO_2 through photosynthesis. To stop photosynthesis, plants were placed in sealed respiration chambers in absence of light during respiration measurement. Phospholipids-fatty-acids (PLFA) analyses were used to determine change in microbial community composition induced by clipping. The possible generalization of changed SOM mineralization after clipping was tested for six gramineae and one leguminous typically present in temperate grasslands. This study aims to find out the effect of grazing-induced change in plant physiology on SOM mineralization. The change in plant community composition and soil processes that results after repetitive grazing (Ward et al., 2007) over years is out of the scope of this study.

2. Materials & Methods:

2.1. Soil sampling and plant sowing:

The soil used in this experiment was sampled from an upland grassland located in the environmental research observatory (ORE) established by the French National Institute for Agricultural Research (INRA) in central France in 2003 (Theix, 45°43'N, 03°01'E). The local climate is semi-continental, with a mean annual temperature of 9°C and an average annual rainfall of 760mm. Before 2003, the site was managed as permanent grassland for more than 50 years (Fontaine et al., 2007). The soil is a drained Cambisol developed from granitic rock. For sampling, the upper 10 cm of soil profile were removed because it is rich in fresh C. Given that respiration of this pre-existing fresh C cannot be separated from that of recalcitrant soil carbon and that the presence of plants can also modify fresh C decomposition (Personeni and Loiseau 2004), it is advisable to use a soil with lower proportion of fresh C in order to determine the effect of plants on recalcitrant SOM. Thus, soil was taken from 10-40 cm soil profile. The fresh soil was sieved at 5 mm and was filled in PVC pots. Each pot was of 40 cm height and 9.8 cm internal diameter and contained 2.87 kg of dry soil. The soil properties are: pH 6.1$\pm$0.21; clay (%) 27$\pm$1.3; soil organic carbon (g kg^{-1} soil) 26.7$\pm$0.37 and soil organic carbon $\delta\ ^{13}C$ (‰) -26.7$\pm$0.02. In April 2009, eight pots were sown with *Lolium perenne* (Lp) at a density of 2000 seeds m^{-2} and four pots were kept bare as control soil (S). Automated drip irrigation method was used for water supply and the water flow was adjusted gravimetrically to keep the soil moisture around 75$\pm$5% of field capacity of soil. All planted pots were fertilized with nitrogen (70 kg N ha^{-1}), phosphorus (100 kg P_2O_5 ha^{-1}) and potassium (200 kg K_2O ha^{-1}) just after first (non-experimental) clipping carried out 122 days after their germination. Plants were allowed to fully develop their roots system during 190 days before the experimental clipping was administered. Four planted pots were clipped 5 cm from soil (clipped treatment, CP). Four planted pots were left intact as unclipped plant control (P).

Carbon dioxide and its isotopic composition released by the plant-soil system were measured for all treatments (S, P and CP) immediately and 30 days after experimental clipping. The effect of clipping on SOM mineralization was determined by comparing respiration from P and CP. Plant biomass, plant C and N content, soluble soil organic C, mineral nitrogen, microbial biomass and PLFA were measured following destructive harvesting of pots 30 days after clipping.

To find if clipping has similar effect on SOM mineralization across dominant species of the site, monocultures of six species namely *Trisette flavescence* (Tf), *Poa trivialis* (Pt), *Festuca arundinacea* (Fa), *Bromus erectus* (Be), *Brachypodium pinnatum* (Bp) and *Trifolium repens* (Tr) were also established. These species were sown and cultivated in exactly the same conditions as the model species *Lolium perenne*. However, the effect of clipping on SOM mineralization was determined by measuring soil respiration in the same pot before and after plant clipping. This approach was adopted to reduce the number of planted pots.

2.2. Labelling system & mesocosm:

The scheme of experimental set-up is shown in figure 1 (Supplementary information). It consisted of two parts: a labelling air production system and a mesocosm containing planted and bare pots. Details on production of labelled air are available in Supplementary Information. Briefly, ambient air was taken into the system by a compressor and its CO_2 and H_2O contents and all other particles were scrubbed by a molecular sieve. The decarbonised air was then mixed with ^{13}C depleted CO_2 of fossil-fuel origin ($\delta\ ^{13}C$: -38.55 $\pm$ 0.07 ‰) and conducted through a humidifier ($1m^3$:$1m^2$ cross corrugated cellulose pads). The water flow in the humidifier was regulated such that the relative humidity of labelled air reaching to mesocosm was maintained around 50-60%. This moistened air had CO_2 concentration of 400 $\pm$ 20 ppm before entering into mesocosm.

The mesocosm was made-up of an iron box with a plexiglass mounted on it. It had dimensions of 350 × 140 × 140 cm. The advantage of using plexiglass is that it does not change the wavelength of sunlight entering into mesocosm. All planted and bare soil pots were placed in mesocosm that was continuously ventilated with air produced by the labelling system. The volume of air in mesocosm was renewed twice a minute so that the unlabelled air respired by soil might not change the labelling signature of air present in the mesocosm. We verified with a smoke apparatus that turbulences in mesocosm were sufficient to ensure the mixing of air. The ventilation of mesocosm also allowed keeping a temperature difference of 1-2°C between inside and outside of mesocosm.

2.3. Respiration measurement:

For all the species involved in the experiment, plant-pots were put back in mesocosm for 24 hours after clipping. Then, pots were taken out from labelling mesocosm and were sealed in air-tight PVC chambers (height 100 cm, diameter 15 cm) for 24 hrs. The absence of light stopped photosynthesis and avoided the plant absorption of soil respired CO_2. Carbon dioxide released by the plant-soil system was trapped in 200 ml of 1M NaOH that was placed in respiration chambers. By conducting additional measurement on respiration chambers by gas chromatography, we quantified that more than 99% of CO_2 respired by the pot (soil + plant) over 24 hours was trapped successfully into NaOH. Total carbon trapped in NaOH was measured with a total inorganic-C analyzer. The ^{13}C abundance of trapped CO_2 was analysed with an Isotope-Ratio Mass Spectrometer (IRMS) after precipitating the carbonates with excess $BaCl_2$ and filtration. The soil-derived CO_2-C (*Rs*, mg CO_2-C kg^{-1} dry soil day^{-1}) was separated from plant-derived CO_2-C (*Rp*, mg CO_2-C kg^{-1} dry soil day^{-1}) using mass balance equations:

$$Rs + Rp = Rt$$

$$Rs \times A_s^{13} + Rp \times A_p^{13} = Rt \times A_t^{13}$$

where A_s^{13} is the ^{13}C abundance (dimensionless) of soil carbon, A_p^{13} the ^{13}C abundance of plant, *Rt* the total CO_2 emitted by the pot (soil *plus* plant) and A_t^{13} its ^{13}C abundance. *Rp* corresponds to CO_2-C coming out from whole plant respiration, mycorrhizae and microbial respiration of rhizodeposits and plant litter.

The rhizosphere priming effect (*RPE*, mg CO_2-C kg^{-1} dry soil day^{-1}) induced by the plant was calculated as:

RPE = (*Rs*, planted soil) – (*Rs*, control soil)

2.4. Soil and plant analyses:

Plant roots were washed to remove soil attached to them. Roots and shoots were then dried at 60°C and finely ground. They were then analysed with an elemental analyser attached to an Isotope-Ratio Mass Spectrometer (IRMS) for total C and N contents and ^{13}C abundance. In order to quantify soil mineral nitrogen, 10 g fresh soil was extracted with 40 ml of 1M KCl for 45 minutes. The extract was filtered and analysed by continuous flow colorimeter. The microbial biomass and its C/N ratio were measured by the fumigation-extraction technique (Vance *et al*, 1987). The extraction of soluble C with K_2SO_4 (30mM) from soil before the fumigation was used to determine soluble organic carbon in soil.

2.5. PLFA measurements:

At the end of the experiment, the pots were destroyed and soil from each pot was thoroughly homogenised. Each sample was freeze-dried, sieved at 2 mm and the remaining plant materials were removed. Phospholipidic fatty acids (PLFAs) were extracted using a modified method of Bligh and Dyer (1959) (Frostegård et al., 1991). Briefly, fatty acids were extracted in a single-phase mixture of chloroform:methanol: citrate buffer (1: 2:0,8, vIvlv, pH 4,0) shaken at 300 rpm for 1H. Phase splitting was obtained by adding equal volume of chloroform and citrate buffer. The organic phase was then submitted to solid phase extraction on silica gel cartridge (Discovery® DSC-Si SPE Tube bed wt. 500 mg, volume 3 mL, from Supelco). Neutral lipids, glycolipids and PLFAs were eluted by chloroform, acetone and

methanol, respectively. Methyl nonadecanoate (fatty acid methyl ester 19: 0) was added as an internal standard and PLFAs were *trans*-methylated under mild alkaline condition to yield fatty acid methyl esters (FAMEs), (Dowling et al., 1986). FAMEs were then analysed by GC/MS (4000 GC/MS, Varian) in split-less mode (1µL, injector temperature: 250°C) equipped with a BPX70 column (60 m, 0.25mm i.d., 0.25mm df. , SGE), and helium as a carrier gas. The temperature program was 50 ◦C for 5 min, raised to 165°C at 15°C/min, followed by a gradient of 2°C/min up to 225°C. This temperature was held for 15min. To identify the FAMEs, the retention times and the mass spectra were compared with those obtained from standards (Bacterial Acid Methyl Ester (BAME) Mix from Supelco and 11 Hexadecenoic acid (92% cis, 8% trans) from Matreya). Gram positive bacterial PLFAs were i15:0, a15:0, i16:0, i17:0 and gram negative baterial PLFAs were 17:0cy, 19:0cy & 16:1ω9. Fungal PLFAs were 18:1ω9c, 18:2ω9t and 18:2ω6c whereas mycorrhizal PLFA was 16:1ω5c. The PLFAs like 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0 were analysed as 'universal' groups i.e. which can be from bacteria as well as fungi.

2.6. Statistical Analyses:

For model species *Lolium perenne*, a repeated-measures ANOVA with post hoc pair-wise comparisons was used to determine the effect of clipping on total respiration (*Rt*), soil-derived CO_2 efflux (*Rs*), plant-derived CO_2 efflux (*Rp*) and rhizosphere priming effect (*RPE*) . The time after clipping was used as random factor. The clipping treatment effect on shoot and root biomasses, N export *via* shoot biomass production, C %, N % and C/N of shoots/roots was determined by paired t-test (95% CI). One way ANOVA was used to determine the effect of treatments (bare soil, clipped or unclipped plants) on soil microbial biomass, their N content & C/N ratios, soluble C in soil and various PLFA contents.

For the generalization experiment, multifactor ANOVA was used to determine the differences in all effluxes i.e. *Rt, Rp, Rs,* and *RPE* with species and clipping as fixed factors. For this

experiment, the effect of clipping was determined by measuring respiration fluxes in the same pot before and after plant clipping. Given the average temperature was different before and after clipping ($\Delta T=3°C$), all respiration fluxes were normalized to a common temperature (20 °C). All statistical tests were performed with Statgraphics Plus (Manugistics, USA)

3. Results:

3.1. Plant biomass and isotopic composition

Plants were successfully labelled with ^{13}C depleted CO_2 and there was no difference between aboveground and belowground isotopic signatures (data not shown). The isotopic signature ($\delta^{13}C$) of different plants varied between -55.12±0.03 ‰ and -57.23±0.07 ‰. The difference between $\delta^{13}C$ of soil and plant was more than -28 ‰ which is sufficient to distinguish plant derived and soil derived effluxes and calculate rhizosphere priming effect.

For model species, *Lolium perenne*, clipping had no significant effect (*p*-value > 0.05) on root biomass. In contrast, shoot biomass in clipped treatment was significantly lower than that in unclipped treatment (*p*-value < 0.001, Figure 1a). Moreover, when shoot biomass harvest of experimental clipping was pooled with that at the end of experiment for clipped treatment, it was significantly lower than the shoot biomass of unclipped treatment (*p*-value < 0.001, Figure 1e). Thus an increased above ground production amounting 1.34±0.42 (g pot^{-1}) was calculated in unclipped treatment as compared to clipped treatment. This corresponded to an additional N export (13.06±6.48 mg N pot^{-1}) by the plant in unclipped treatment (*p*-value < 0.05, Figure 1f,). There was no effect of clipping on N contents (%) and C/N ratio of shoot or root biomasses (*p*-value < 0.05, Figure 1b, c &d).

3.2. Respiration fluxes:

For model species, *Lolium perenne*, clipping caused rapid (within 48 hours) and significant decrease in total respiration (*p*-value <.0.001). This decrease persisted for 30 days after

clipping (Table 1). Moreover, this decrease in *Rt* was a result of significant decreases in both of its components i.e. *Rp* the respiration from living plant and microbial respiration of labelled plant rhizodeposits and *Rs* the microbial respiration of SOM.

Irrespective of clipping, the presence of plants significantly accelerated the SOM mineralization (*Rs*) when compared to bare soil (Table 1) indicating a rhizosphere priming effect (*RPE*). *RPE*, calculated by subtracting *Rs* in bare soil from *Rs* in planted soil, was almost halved (54% of that under unclipped) by clipping and this decreased rate of *RPE* under clipped Lp persisted 30 days after clipping (Table 1, Figure 2a, *p*-value < 0.001).

In the generalization experiment, clipping was found to significantly (*p*-value < 0.05) reduce the total respiration within 48 hours in all grassland species (Table, 1). Again this decrease in *Rt* was the outcome of significant decreases in both of its components i.e. *Rs* and *Rp*. Irrespective of clipping, all the grassland species induced significant acceleration of SOM mineralization i.e. *RPE.* Clipping caused a significant decrease in *RPE* in all the grassland species (*p*-value < 0.001, Figure 2 b). However the quantitative response of different species *vis-à-vis* decrease in *RPE* after clipping was different. The minimum decrease was shown by *Poa trivialis* (*RPE* after clipping was 80% of that before clipping) and the maximum decrease occurred in *Brakipodium pinnatum* (*RPE* after clipping was 44% of that before clipping).

3.3. Soluble C & mineral nitrogen in soil:

An increasing gradient for organic soluble C in soil was found from bare to unclipped to clipped treatments (Figure 3b). However the difference in soluble C contents was only significant between bare and clipped treatments.

All the mineral nitrogen present in soils was in the form of nitrate. Mineral nitrogen contents in soil were significantly higher in clipped than unclipped treatment (Figure 3d). Bare soil showed significantly higher mineral N contents as compared to clipped and unclipped-plant treatments.

3.4. Microbial biomass & community composition:

Clipping did not modify the microbial biomass or its C/N ratio (p-value > 0.05, Figure 3a& c). Mean relative contribution of a saprophytic fungal group (*18:2ω6c*) to total PLFA increased significantly in the presence of plants. However their relative abundance was significantly lower in clipped than unclipped treatment (p-value <0.05, Table 2). Mean relative contribution of arbuscular mycorrhizal fungi (AMF, *16:1ω5c*) to total PLFA increased due to planting but showed no effect of clipping. A gram positive bacterial group (*i16:0*) was increased (p-value < 0.05) by clipping. All other microbial groups showed no effect of clipping or planting.

4. Discussion:

Thanks to an original method that allows quantifying the rhizosphere priming effect induced by grassland species, we show that clipping reduces recalcitrant SOM mineralization and total respiration in soil-plant system. This result is common to six gramineae and one leguminous species typical of temperate grasslands (Figure 2). The decrease in SOM mineralization after plant clipping can explain significant reductions in total soil respiration from ecosystems exposed to clipping and grazing (Bremer et al., 1998; Craine et al., 1999, Wan & Luo, 2003; Cao et al., 2004; Bahn et al., 2006) despite the acceleration of plant litter decomposition (Klumpp et al., 2009). Hamilton & Frank (2001) suggested an increased SOM mineralization after clipping based on increased microbial biomass, soluble C and mineral N in soil in their study. Like them, we found an increased soluble C and mineral N in soil; however, our results do not support the idea that clipping could accelerate the SOM mineralization. Thus our results reconcile the apparent contradiction between results of experiments measuring gas emissions suggesting decrease in SOM mineralization after clipping (e.g. Bahn et al., 2006)

and the results of experiments measuring soluble C, microbial biomass and mineral N in soil suggesting an increase in SOM mineralization after clipping (e.g. Hamilton and Frank., 2001).

The increased availability of labile C in the soil does not necessarily result in accelerated SOM mineralization because it depends upon which microbial groups are stimulated by additional C supply (Fontaine et al., 2003). Our results show that the presence of plants and clipping induced changes in microbial community structure (Table 2). The presence of plants stimulated the abundance of mycorrhizal and saprophytic fungi in parallel with *RPE*. However, the clipping, which induced a strong decrease in *RPE*, specifically reduced the abundance of saprophytic fungi. These results support the idea that the fungi play a key role in recalcitrant SOM mineralization (*RPE*) (Fontaine et al., 2011). Moreover, clipping increased the abundance of gram positive bacteria as shown elsewhere (Bardgett et al., 2001; Klumpp et al., 2009). It has been shown that the proliferation of gram positive bacteria after clipping induced an acceleration of plant litter decomposition. These results suggest that clipping modified the competition between saprophytic fungi and gram positive bacteria for plant C and favoured the preservation of old SOM.

How the decrease in *RPE* and changes in microbial community structure after clipping can be explained? Our results suggest that clipping induced a cascade of effects on plant functioning, soil processes and microbial community. Clipping reduced the production of shoot biomass thus reduced export of N by the plants (Figure 1). This is explained by reduced leaf area which in turn curtails total plant photosynthesis, transpiration and uptake of mineral nitrogen (Macduff 1992). The reduced N uptake by plant led to an increase in mineral N availability (Fig 1) for soil microorganisms, especially for soil bacteria which are typically limited by nitrogen. Given bacteria and fungi are in competition for plant C acquisition, the nitrogen-induced stimulation of bacteria suppresses fungal populations and thereby *RPE*.

Our study shows strong link between above ground biomass, plant N uptake and SOM mineralization which may help synchronizing plant photosynthesis and availability of mineral N in soil. When plant is carrying out high amounts photosynthesis in the presence of favourable light intensity (e.g. summer season in temperate grasslands), its N uptake exceeds than the soil offer in terms of mineral N resulting in decreased availability of soil mineral N and increased fungi : bacteria ratio. These changes accelerate SOM mineralization which liberates N held up in the recalcitrant SOM stocks. On the other hand, when total photosynthesis by plant is reduced due to clipping or low light intensity, its N uptake is less than the soil offer in terms of N. The accumulation of mineral N in soil decreases SOM mineralization leading to sequestration of mineral N in SOM stocks. If this excess of nutrients is not sequestered, they would be leached or denitrified. Therefore, our study supports the idea that soil ecosystem works as a bank of nutrients for the plant maximizing plant productivity, nutrient retention and C sequestration in the long term. Given that soil cultivation have severely reduced the microbial populations as a result of reduced incorporation of plant C and physical disturbance of soil, this bank mechanism is strongly limited explaining the leaky nutrient cycling and addiction to mineral fertilizers of cultivated soils. Developing grain and forage production system based on permanent plant cover that will strengthen the bank mechanism could be key step towards an agriculture ensuring food production and environmental services like C storage and preservation of groundwater quality.

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Figure Captions:

Figure 1. Plant biomass (a) C contents (%) (b) N contents (%) (c) C/N ratios (d) shoot biomass production since experimental clipping till end of experiment (e) N exported *via* shoot biomass for clipped and unclipped treatments in *Lolium perenne* (f) (*** p-value < 0.001, * p-value < 0.05).

Figure 2. *RPE* for unclipped and clipped treatments 1 and 30 days after clipping for model species, *Lolium perenne* (a) *RPE* for all species in generalization experiment before and after clipping (*** p-value < 0.001)

Figure 3. Microbial biomass (a), soluble C (b), microbial C/N ratio (c) and mineral N in unclipped, clipped and bare soil treatments (d) (One way ANOVA, p-value < 0.05 when significant)

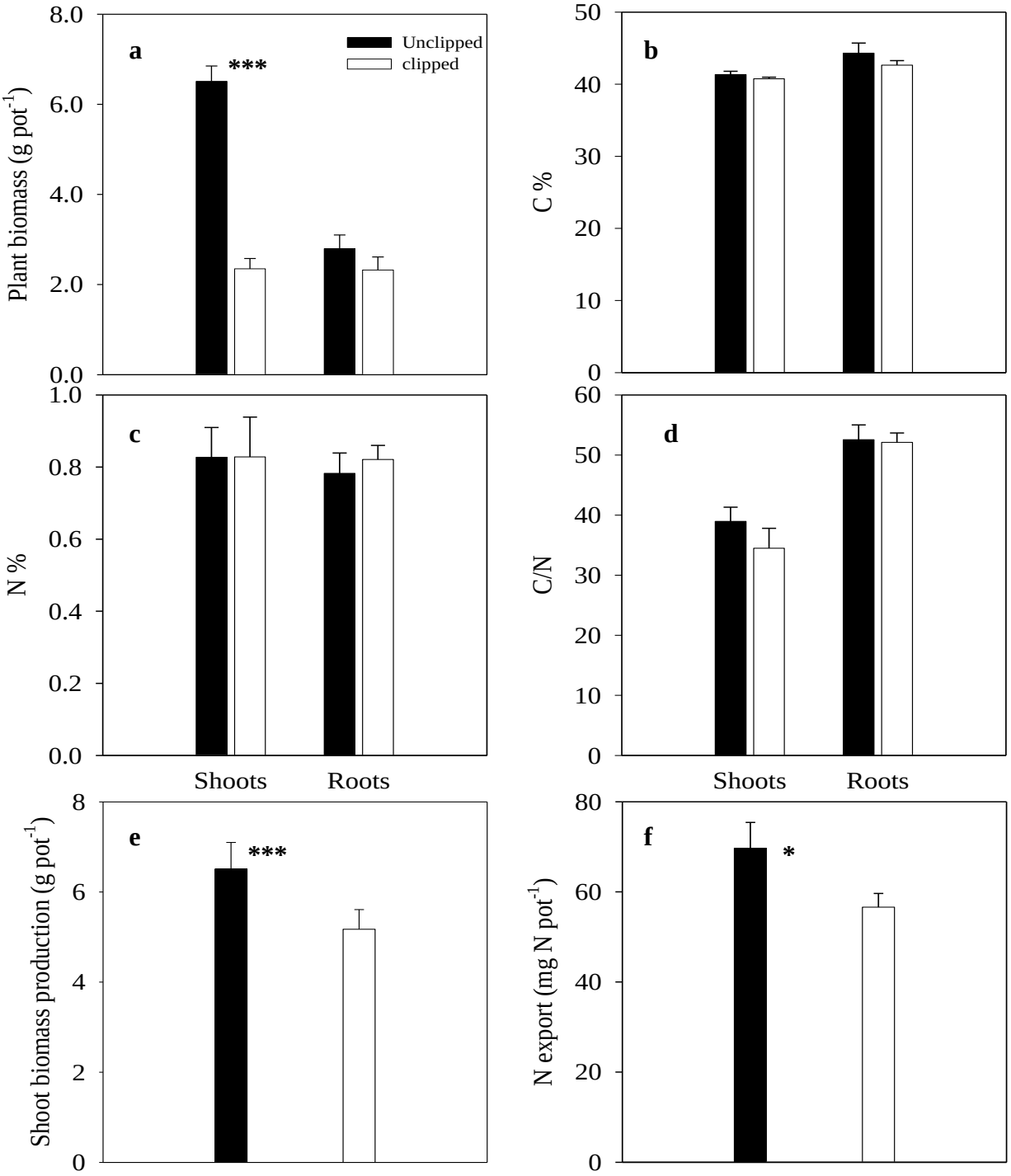
a
***
Unclipped
clipped
Plant biomass (g pot-1)
8.0
6.0
4.0
2.0
0.0
b
C %
50
40
30
20
10
0
c
N %
1.0
0.8
0.6
0.4
0.2
0.0
d
C/N
60
50
40
30
20
10
0
Shoots
Roots
Shoots
Roots
e
***
Shoot biomass production (g pot-1)
8
6
4
2
0
f
*
N export (mg N pot-1)
80
60
40
20
0

Figure 1.

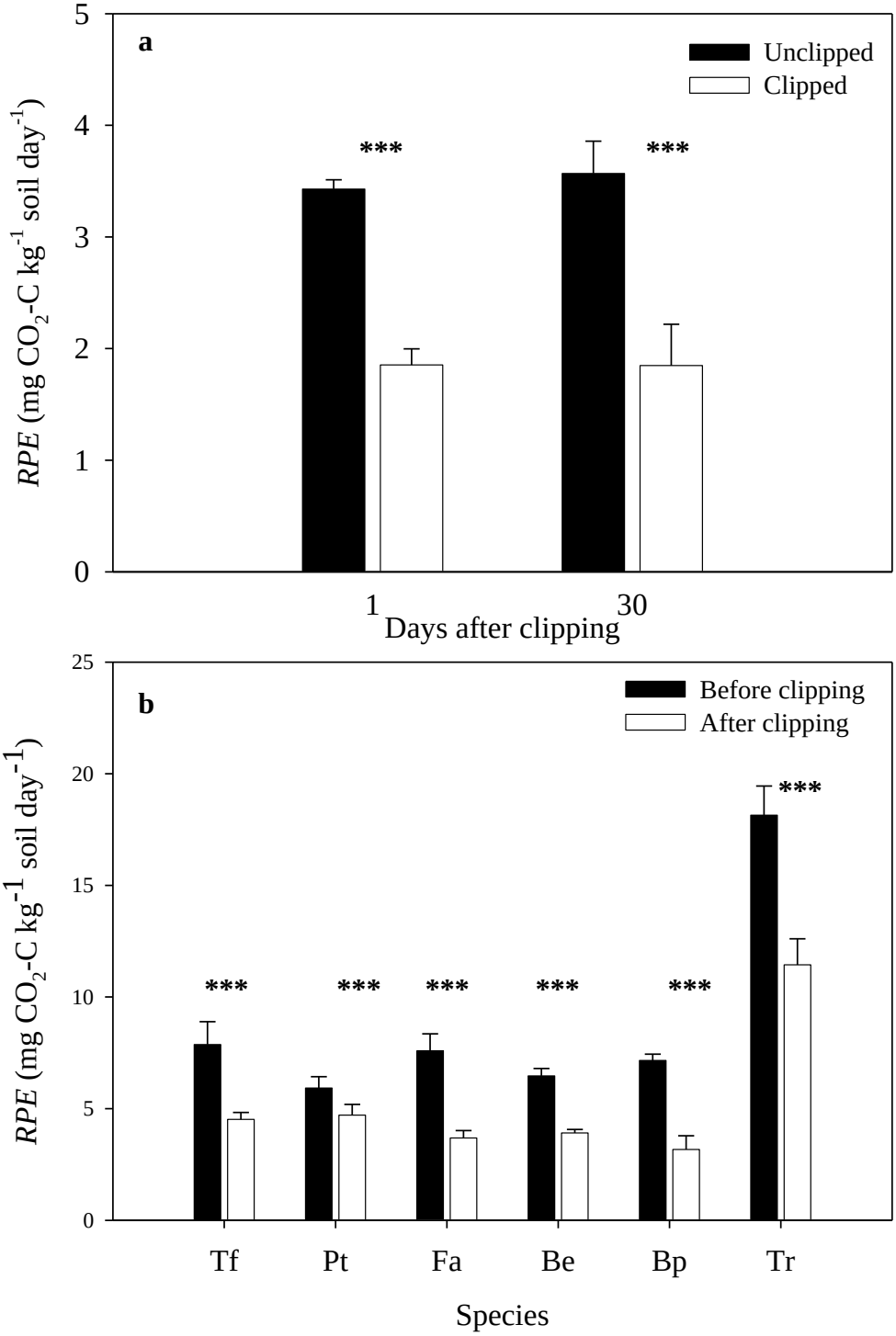
a
Unclipped
Clipped
***
***
RPE (mg CO2-C kg-1 soil day-1)
0
1
2
3
4
5
1
30
Days after clipping
b
Before clipping
After clipping
***
***
***
***
***
***
RPE (mg CO2-C kg-1 soil day-1)
0
5
10
15
20
25
Tf
Pt
Fa
Be
Bp
Tr
Species

Figure 2.

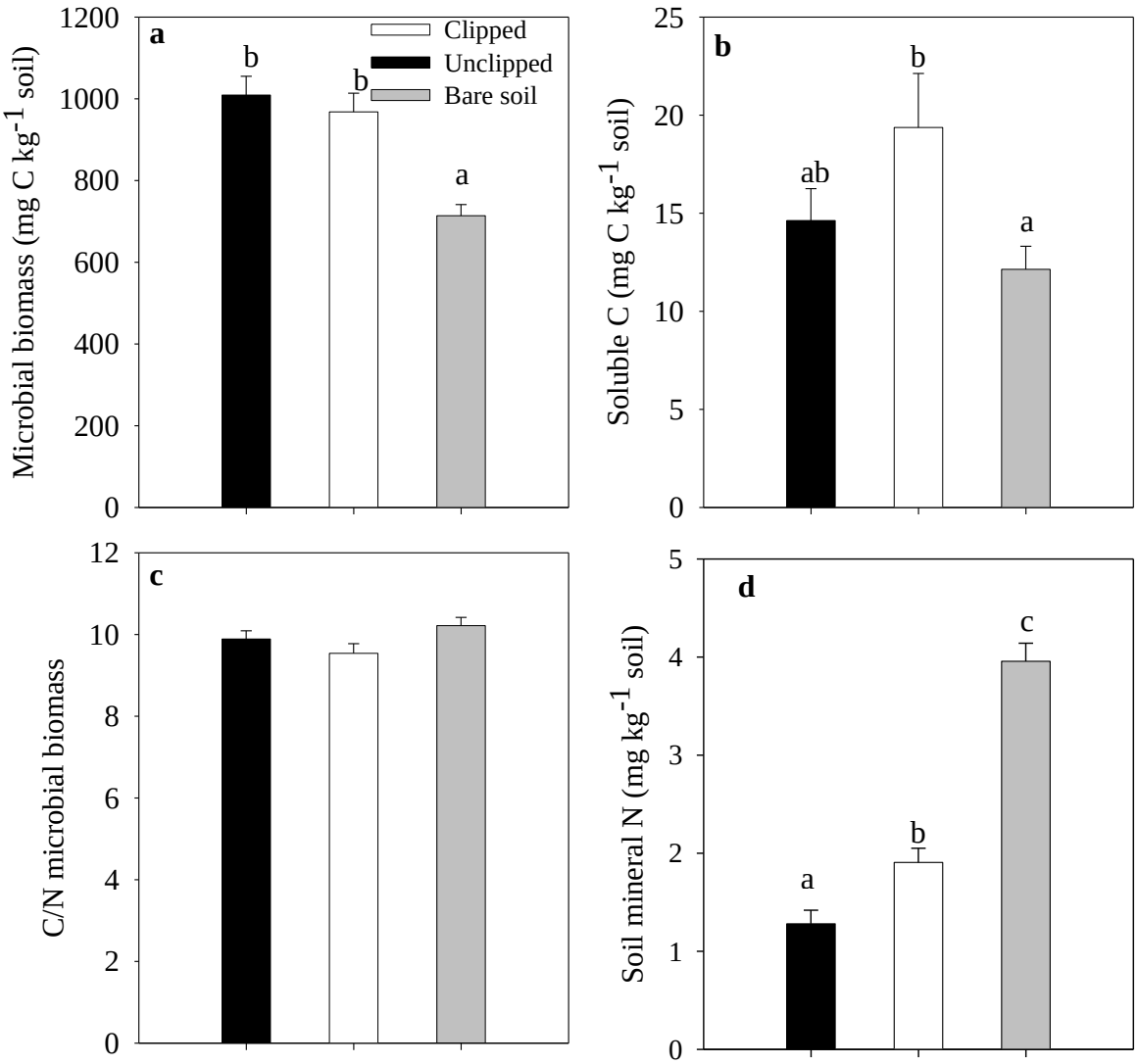
a
b
Clipped
Unclipped
Bare soil
Microbial biomass (mg C kg-1 soil)
1200
1000
800
600
400
200
0
b
a
b
Soluble C (mg C kg-1 soil)
25
20
15
10
5
0
ab
b
a
c
C/N microbial biomass
12
10
8
6
4
2
0
d
Soil mineral N (mg kg-1 soil)
5
4
3
2
1
0
a
b
c

Figure 3.

Table 1: Effect of clipping on different respiration fluxes of model species *Lolium perenne* and other temperate grassland species as affected by clipping.

Rt = total CO_2-C, *Rp* =plant-derived CO_2-C, *Rs* = soil-derived CO_2-C,), *RPE* = rhizosphere priming effect. *p*-value < 0.05, Values are means (n = 4).

Experiment	**Species**	**Treatment**	**CO_2-C efflux (mg CO_2-C kg^{-1} soil day^{-1})**			
			Rt	***Rp***	***Rs***	***RPE***
Model species	*Lolium perenne*	***1 Day after Clippinig***				
Experiment		Unclipped	16.93 b	8.84 b	7.7 c	3.43 b
		Clipped	11.04 a	4.28 a	6.13 b	1.85 a
		Bare soil			4.27 a	
		30 Days after Clippinig				
		Unclipped	13.6 b	6.6 b	7 c	3.57 b
		Clipped	7.56 a	2.27 a	5.28 b	1.85 a
		Bare soil			3.44 a	
Generalization	*Trisette flavescence*	Before clipping	29.44 b	16.62 b	12.82 b	7.87 b
experiment		After clipping	19.47 a	10.66 a	8.82 a	4.52 a
	Poa trivialis	Before clipping	20.80 b	9.92 b	10.87 b	5.93 b
		After clipping	14.72 a	5.71 a	9.00 a	4.71 a
	Festuca arundinacea	Before clipping	26.64 b	14.11 b	12.53 b	7.59 b
		After clipping	17.72 a	9.77 a	7.98 a	3.68 a
	Bromus erectus	Before clipping	24.29 b	12.88 b	11.41 b	6.47 b
		After clipping	16.24 a	8.03 a	8.21 a	3.91 a
	Brachypodium pinnatum	Before clipping	24.25 b	12.15 b	12.10 b	7.16 b
		After clipping	13.40 a	5.94 a	7.46 a	3.17 a
	Trifolium repens	Before clipping	112.21 b	85.31 b	26.90 b	18.15 b
		After clipping	40.86 a	23.64 a	17.22 a	11.44 a

Table 2. Mean relative abundance (mol PLFA C %) of individual biomarker PLFAs in response to experimental treatments (One way ANOVA, p-value < 0.05), NS, Non-significant.

Community	PLFA	Un-clipped	Clipped	Bare
Fungal	*18:1ω9t*	3.38 [a]	3.40 [a]	3.85 [a]
	18:1ω9c	14.04 [a]	12.96 [a]	14.51 [a]
	18:2ω6c	**3.99 [c]**	**2.51 [b]**	**1.50 [a]**
AMF	*16:1ω5c*	**3.72 [b]**	**3.68 [b]**	**2.65 [a]**
Gram (-)	*17:0cy*	7.29 [a]	7.36 [a]	7.91 [a]
	19:0cy	1.39 [a]	1.32 [a]	1.64 [a]
	16;1ω9c	5.11 [a]	6.06 [a]	6.55 [a]
Gram (+)	*i15:0*	6.74 [a]	7.57 [a]	6.69 [a]
	a15:0	7.21 [a]	7.78 [a]	7.30 [a]
	i16:0	**6.87 [a]**	**7.46 [b]**	**7.45 [b]**
	i17:0	5.00 [a]	4.45 [a]	5.35 [a]
Universal	*14:0*	0.55 [a]	0.55 [a]	0.38 [a]
	15:0	0.80 [a]	0.86 [a]	0.69 [a]
	16:0	26.19 [a]	26.23 [a]	24.91 [a]
	17:0	1.06 [a]	1.08 [a]	1.05 [a]
	18:0	6.01 [a]	6.07 [a]	6.80 [a]
	20:0	0.66 [a]	0.66 [a]	0.77 [a]