

# Soil functioning in a mosaic of herbaceous communities of a chalky environment: temporal variations of water availability and N dynamics

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## Abstract

**Background and aims** Although changes in water and nitrogen (N) supply have been largely used to explain modifications in plant communities, the spatio-temporal variability of those factors has been little studied in chalky environments.

**Methods** In this study, we explored for 1 year the temporal variations in soil water content, N inorganic forms and net N-mineralization and nitrification for two horizons in three herbaceous communities (short grasslands, tall grasslands, and encroached grasslands) in the Hénouville Nature Reserve (Upper-Normandy, France). Plant available soil water and permanent wilting points of seven plant species were also characterized.

**Results** We found that plant available soil water was lower in short grasslands than in tall grasslands and encroached grasslands. Soil water content was below permanent wilting point during four months in short grasslands and only three months in the other communities. Seasonal patterns for inorganic N content

and N-mineralization and nitrification were observed with peaks of  $\text{NH}_4^+\text{-N}$  in summer and peaks of N-mineralization in spring.

**Conclusions** For the studied year, our data highlight the harsh soil desiccation that vegetation endured during the late spring (active growth period) and summer, and show that water shortage is an ecological factor affecting the N cycling in the three successional herbaceous communities.

**Keywords** Water availability · Inorganic N content · N-mineralization and nitrification · Chalk hillside

## Introduction

It is widely recognised that the availability of soil resources strongly affects the floristic composition, structure and productivity of plant communities (Tilman 1988). It is also accepted that belowground resources vary both spatially and temporally (Ryel et al. 1996). These variations in belowground resources depend on climatic conditions (precipitation and temperature), soil properties (soil types, organic matter and clay contents), plant effects (root uptake, litter input) and soil organisms activity (macrofauna, mesofauna, microfauna and microbial activity) (Lavelle and Spain 2001). In addition, the availability of some of these resources may be restricted in natural environments, causing severe stress for vegetation (Chapin 1991). This stress

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often results from water and nitrogen shortage which dramatically limits plant growth (Lambers et al. 1998).

Water is a limiting resource for biomass production in most terrestrial ecosystems (Huxman et al. 2004). It is also a determinant factor governing the structure and dynamics of plant communities (Singh et al. 1998). Water availability exhibits a high degree of spatial and temporal variability (Schneider et al. 2011) and depends on climatic constraints (precipitation, temperature), topography (slope, exposure), soil properties (texture, structure, bulk density, organic matter and clay content, soil depth) and vegetation characteristics (plant cover, canopy, plant traits) (Ruiz-Sinoga et al. 2011). Plants may also be subjected to periods of soil water deficit, which can have severe impact on their survival. For instance, during a drought, many plants could survive below the permanent wilting point of soil, but all would die when they can no longer extract the small amount of remaining water held tightly to soil particles (Rodríguez-Iturbe and Porporato 2004). Furthermore, when drought events are recurrent, only water stress-adapted plants persist. However, when they are occasional, then drought-sensitive plants/individuals are eliminated and possibly recolonize later (Buckland et al. 1997). In dry habitats, water availability may also influence nutrient supply to vegetation by controlling nutrient fluxes and biogeochemical cycles (Schimel et al. 1997). In particular, the soil water dynamic is related to nitrogen cycling through its effects on nitrogen availability (Giese et al. 2011) and mineralization rate (Schimel and Parton 1986).

Nitrogen (N) is usually mentioned as a factor affecting plant growth (Lambers et al. 1998) and vegetation dynamics (Tilman 1988). It is one of the most strongly limiting nutrients for primary production in terrestrial ecosystems and in contrast to other nutrients, its availability commonly increases during secondary succession (Vitousek et al. 1989). N dynamics also depend on biological fixation, internal cycling, denitrification, leaching, fertilization and atmospheric deposition (Galloway et al. 2004). These processes are usually spatially and temporally structured and are influenced by abiotic factors such as climatic and edaphic variables (Robertson et al. 1988). Temperature, soil water content (SWC), pH, quality, abundance and accessibility of organic matter are the main factors influencing N cycling (Booth et al. 2005). Furthermore, biotic factors also regulate N cycling through feedback exerted by dominant plant

species (Wedin and Tilman 1990), changes in quality and quantity of litter (Pastor et al. 1987), activities of soil fauna and microflora (Decaëns et al. 1999; Ekschmitt et al. 1999; Dunn et al. 2006), root and mycorrhiza (Milleret et al. 2009) or grazing effect (Xu et al. 2007).

There is little ecological literature focusing on both the variations in soil N and water availability in relation to vegetation changes (Kalicka et al. 2004). However, water-nitrogen-vegetation systems are characterized by many interrelationships that justify studying all these components simultaneously. In particular, Grime and Curtis (1976) highlighted the link between nutrient stress and drought for determining plant composition in calcareous grasslands and Leuschner (1989) noticed that water shortage was a limiting factor influencing N cycling in these ecosystems. Similarly, Davy and Taylor (1974a) and Ellenberg (1977) showed that N mineralization and nitrification were greatly reduced when chalk soils dried in summer. Moreover, it has been mentioned that low soil water availability prevented the development of competitive grass populations (Thompson and Grime 1988). In contrast, it has been demonstrated that the impact of atmospheric pollution by N resulted in the gradual increase of social gramineous species in most calcareous grasslands of West European countries (Bobbink and Willems 1987; Bobbink et al. 1998, 2010).

In Atlantic Europe, calcareous grasslands benefit from an oceanic climate with a relatively homogeneous distribution of rainfall throughout the year (Duckworth et al. 2000). Paradoxically, they are periodically water-limited due to the specificity of their underlying soils which present rapid drainage and reduced water storage (Kalicka et al. 2008). Although the amount of available water for plants is greater in chalk soils than in sandy or clayey soils (Davy and Taylor 1974b; Smith 1980), soil constraints in those environments may be occasionally significant, especially in summer and sometimes in spring (Buckland et al. 1997). Drought periods may last several days and cause severe dry conditions exposing vegetation to high surface temperatures up to 30 °C in sun (Bennie et al. 2008). Topography and exposure may also be regarded as decisive factors for influencing water supply in chalk soils (Bennie et al. 2006). Due to their location on sunny steep slopes and warm areas, calcareous grasslands present a typically dry and hot microclimate which also favours soil water losses by increasing soil desiccation and evaporation (Bennie et

al. 2008). Furthermore, shallow soils that usually characterize calcareous grasslands influence the availability of water and soil fertility, playing thus a major role in controlling the outcome of species interactions and ultimately affecting the course of succession in those environments (Alard et al. 2005).

Over the last century, agropastoral activities that traditionally characterized chalk hillsides have considerably declined in Northern France, resulting in substantial vegetation changes. The result of secondary succession is a mosaic of plant communities varying in their floristic composition and species dominance (Moog et al. 2002) and corresponding to different successional stages from short grasslands to tall grasslands and shrubs (Dutoit and Alard 1995). According to Alard et al. (2005), this vegetation mosaic reflects differences in the rate of secondary succession resulting from the effects of past land use and local soil conditions. In the same study, they demonstrated the importance of soil physico-chemical properties (soil depth, organic carbon, total nitrogen) to explain the dynamics of vegetation mosaics. However, the study of changes over space and time of the soil resources for plants (water, nitrogen) in this vegetation patchwork remains an open question. The aim of this study was thus to assess the spatial and temporal variations of two indicators of present-day soil functioning (water retention and N dynamics) in different successional stages of a chalk hillside. For this, we measured available soil water for plants (soil water retention capacity, permanent wilting point of soil) and explored for 1 year the variations in SWC, N inorganic forms and N transformations (net N-mineralization and nitrification) in three herbaceous communities. We hypothesized that: (1) there would be differences in soil water availability for plants between communities, (2) there would be differences in soil water regime (seasonal variations in soil water content) between communities and (3) there would be differences in N dynamics between communities.

## Material and methods

### Study site

The Hénouville Nature Reserve (12.2 ha, 49°29'N; 0°56'E) is located in the valley of the Seine River, about 15 km northwest of Rouen (France). The site was used

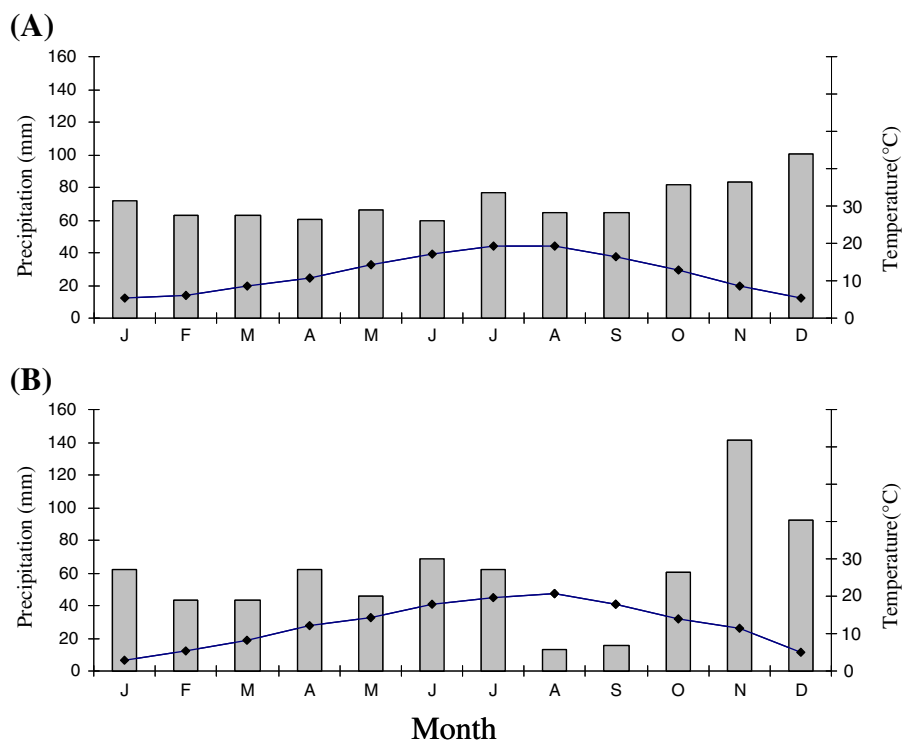
as cultivated fields and vineyards in the Middle Ages and was extensively grazed by sheep and cattle until the middle of the 20th century and was then gradually abandoned in the 1960s. Since 1994, it has been managed by the Upper Normandy conservatory of natural sites with extensive grazing (three cows during three weeks in March) to limit shrub establishment and preserve the vegetation mosaic in its present state. It is located on a steep slope (15 to 40° inclination, 15–75 m elevation) with south-west exposure. Soils are classified as Rendzic Leptosols (IUSS Working Group, WRB 2006), also referred as RENDOSOL according to the “Référentiel Pédologique” (Baize and Girard 2009). They are characterized by a clayey-silty texture and biomacrostructured Aca and AcaCca horizons (noted subsequently as A and AC horizons) overlaying the soft Upper Chalk bedrock (Santonian and Coniacian stages containing 95–99.7 % calcium carbonate and 0.3–5 % clay, Laignel (2003)). Mean annual temperature is  $12.0 \pm 0.7$  °C and annual rainfall is  $854 \pm 137$  mm (METEO FRANCE 1991–2010, Fig. 1a). During the study period (from January to December 2009), precipitations and mean air temperature were 709 mm and 12.4 °C, respectively (Fig. 1b).

### Herbaceous community characteristics

The vegetation of the study site appears as a mosaic of grasslands, shrub thickets and woodlands (Dutoit and Alard 1995, Chabrierie et al. 2002). Despite the relative importance of thickets and wooded stands in the vegetation of the hillside, we restricted our study to herbaceous communities because they present the highest number of rare and protected species, and accordingly are of prime concern in conservation-management planning. We thus selected the three herbaceous communities that are representative of the early vegetation stages of secondary succession as described by Dutoit and Alard (1995):

- (1) Short Grasslands (SG) are characterized by a vegetation height of less than 10 cm, a plant cover less than 75 % and by bare soil patches. Amount of aboveground biomass and plant litter are  $345 \text{ g m}^{-2}$  and  $54 \text{ g m}^{-2}$ , respectively. Soils only consist of a thin (<10 cm), A horizon directly overlying the chalk bedrock (Table 1). Vegetation is typically xeroheliophilous and dominated by oligotrophic dwarf shrubs (*Helianthemum nummularium*, *Teucrium chamaedrys*, *Hippocrepis*

**Fig. 1** Monthly precipitation (bars) and mean temperature (curves) for the 1991–2010 period (a) and for 2009 (b)



*comosa*), accompanied by annuals and grasses (*Linum catharticum*, *Euphrasia stricta*, *Catapodium rigidum*, *Koeleria pyramidata*, *Bromus erectus*).

- (2) Tall Grasslands (TG) are typically covered by a closed vegetation dominated by graminoid species (*Brachypodium pinnatum*, *Sesleria caerulea*, *Carex flacca*, *Festuca lemanii*). Vegetation height ranges from 10 to 20 cm, with aboveground biomass and plant litter of  $510 \text{ g m}^{-2}$  and  $340 \text{ g m}^{-2}$ , respectively. Soils are deeper than in SG (15–20 cm) and consist in an A horizon and a fragmented carbonated AC horizon (Table 1).
- (3) Encroached Grasslands (EG) correspond to a mixed shrub-grass vegetation where mesotrophic species (*Dactylis glomerata*, *Poa pratensis*) are more frequent and bushes (*Cornus sanguinea*, *Prunus spinosa*) of 1–30 cm tall compete with the dominant species *B. pinnatum*, *C. flacca* and *T. chamaedrys*. Vegetation height ranges from 20 to 35 cm, plant biomass is up to  $620 \text{ g m}^{-2}$ , and litter mass is up to  $330 \text{ g m}^{-2}$ . Soils are slightly deeper than in TG (20–25 cm) and present an A horizon and a fragmented carbonated AC horizon (Table 1). The plant species nomenclature follows Tutin et al. (2001).

#### Sampling design

Nine sampling points were taken haphazardly in each community at a minimum distance of 15 m apart to avoid spatial autocorrelation (Alard et al. 2005), giving a total of 27 sampling points (3 herbaceous communities  $\times$  9 sampling points).

#### Soil water properties

Every month, from January to December 2009, 27 soil samples ( $25 \times 25 \times 20$  cm deep) were dug out with a spade, thickness of A and AC horizons was measured, and then each horizon was carefully separated in TG and EG. In total, 45 soil samples (9 sampling points  $\times$  1 horizon for SG, 9 sampling points  $\times$  2 horizon for TG and 9 sampling points  $\times$  2 horizon for EG) were sieved at 2 mm and soil water content (SWC) was measured gravimetrically by drying samples at  $105^\circ \text{C}$  for 72 h (Jarrel et al. 1999).

Available soil water for plants was measured on the 45 soil samples previously sieved at 2 mm and air-dried during three months. Water retention measurements were done with a pressure plate apparatus (Klute 1986). Soil samples ( $7 \text{ cm}^3$ ) were saturated with deionised water during 24 h and then put in pressure extractors (Modele n°1600 and n°1500, Soil

**Table 1** Soil characteristics (mean values±SE,  $n=9$ ) in the two horizons (A and AC) and the three herbaceous communities. *SG* short grasslands, *TG* tall grasslands, *EG* encroached grasslands.Different letters indicate significant differences at the probability level of 0.05 (Tukey HSD and Student's *t*-test)

Variables	Unit	A horizon			AC horizon	
		SG	TG	EG	TG	EG
Soil properties						
Layer horizon	cm	7.4±1.5 a	8.9±1.2 b	9.1±1.2 b	8.9±1.4 a	9.1±1.5 a
pH water	–	7.72±0.14 a	7.85±0.10 a	7.85±0.09 a	7.86±0.10 a	7.84±0.06 a
CaCO <sub>3</sub>	kg m <sup>-2</sup>	30.24±5.18 a	40.26±8.93 a	29.23±10.05 a	–	–
Corg	kg m <sup>-2</sup>	4.79±1.05 a	3.47±0.88 b	3.69±0.91 b	3.34±0.96 a	3.14±0.66 a
Ntot	kg m <sup>-2</sup>	0.45±0.09 a	0.35±0.08 b	0.39±0.09 a	0.35±0.10 a	0.34±0.06 a
Potential N-mineralization rate <sup>a</sup>	%	0.47±0.29 b	1.51±0.30 a	2.13±0.31 a	–	–
C/N	–	11.0±3.3 a	10.2±2.6 a	9.6±2.2 a	10.1±2.7 a	9.3±1.2 a
POlsen	g m <sup>-2</sup>	1.87±0.56 a	1.43±0.28 a	1.53±0.21 a	1.49±0.27 a	1.44±0.26 a
Ca <sup>2+</sup>	kg m <sup>-2</sup>	0.52±0.12 a	0.35±0.08 b	0.41±0.10 a	0.33±0.07 b	0.43±0.11 a
K <sup>+</sup>	g m <sup>-2</sup>	10.13±4.41 a	7.15±2.78 a	6.48±3.10 a	4.71±1.21 b	5.11±2.17 a
Mg <sup>2+</sup>	g m <sup>-3</sup>	8.78±3.70 a	5.58±1.36 a	8.03±2.36 a	4.03±1.24 a	5.54±1.57 a
Cationic exchange capacity (CEC)	cmol + / kg	40.7±10.1 a	26.4±6.3 b	32.2±8.4 a	20.6±5.0 b	28.9±7.0 a
Granulometry						
Sand	%	22.9±5.7 a	23.1±6.4 a	28.5±9.0 a	–	–
Silt	%	42.0±4.6 a	41.9±6.9 a	37.5±6.9 a	–	–
Clay	%	35.1±1.3 a	35.0±1.4 a	34.0±2.1 a	–	–
Soil bulk density <sup>b</sup>	g cm <sup>-3</sup>	0.57±0.05 a	0.59±0.10 a	0.66±0.08 a	0.69±0.07 a	0.64±0.08 a

<sup>a</sup> Potential N-mineralization rate was measured with laboratory incubations using the protocol developed by Hart et al. (1994)<sup>b</sup>  $n=5$ 

Moisture Equipment Corporation, Santa Barbara, California, USA) to collect water under 8 different pressures (i.e. 0.33, 1, 2, 3, 4, 5, 10 and 15 bars). Available soil water for plants corresponded to the difference between water content at soil retention capacity (0.33 bar) and at permanent wilting point (15 bars). The amount of available water was calculated by multiplying water mass (g water g<sup>-1</sup> dry soil) by soil bulk density (g m<sup>-3</sup>) and horizon thickness (cm). Five measures of bulk density of soil surface (A and AC horizons) were carried out using a 100 cm<sup>3</sup> soil corer within each community. The soil cores were dried at 105 °C for 72 h (Blake and Hartge 1986). Soil sieving may greatly influence water retention because soil structure is damaged and aggregates are broken up, and thereby plant available soil water is underestimated (Jarrel et al. 1999). Conversely, when rock fragments of chalk soils are neglected, the available soil water is overestimated (Cousin et al. 2003). Therefore, it was decided to measure water retention properties

on sieved soils to homogenize samples and because the soil used to measure the permanent wilting point of plants was also sieved (Mathieu and Pieltain 1998).

Permanent wilting point of seven plant species (*F. lemanii*, *C. flacca*, *B. pinnatum*, *H. comosa*, *T. chamaedrys*, *H. nummularium* and *S. caerulea*) was measured according to the biological method (Mathieu and Pieltain 1998). These species were chosen because they were present in the three herbaceous communities (Dujardin et al. 2011). Seeds were collected in the field in 2008 and stored at room temperature until sowing in the following spring. Plants were grown from these seeds in cylindrical plastic pots (20 cm diam. × 25 cm depth) on a soil that was previously collected in the three herbaceous communities (pooled A and AC horizons) and sieved at 5 mm. In spring, a total of 35 plants (5 replicates per species) were cultivated under full light conditions and watered regularly. In autumn, plants were placed in a greenhouse, watering was stopped and plant water status was tested by



measuring leaf water content. One leaf per plant was cut and immediately weighed to determine fresh weight. Leaves were then dried at 65 °C for 72 h and the dry weight was determined. As soon as plants showed first signs of wilting, another leaf was taken and the same protocol was applied to check plant water status. We considered that plants were definitively dead when leaf water loss exceeded 50 % compared with saturated leaf water content (Nagase and Dunnett 2010). We suspected that *S. caerulea* was the more xerophilous plant and we referred to Dixon (1986) who demonstrated that leaves of this species could reach a relative water content of 36 % and then recover. We thus considered that *S. caerulea* was dead when relative water content was below this threshold value. Once we were sure that plants had definitively wilted, three soil samples per pot were taken and dried at 105 °C for 72 h to measure SWC (Jarrel et al. 1999).

A complementary measurement was performed in the field in order to evaluate the soil water content changes after a significant rainfall event that occurred in May 26th, 2009 (12 mm of rain). Every two days, 18 soil samples were taken randomly in the A horizon of each herbaceous communities with a steel auger (3.5 cm diam.) from May 27th, 2009 (one day after rainfall) to June 7th, 2009 (before a new rain event). All soil samples were sieved at 2 mm and SWC was measured by drying samples at 105 °C for 72 h (Jarrel et al. 1999).

### Soil N dynamics

Temporal patterns of inorganic N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) and N transformations (net N-mineralization and nitrification) were investigated during 1 year (2009) for the different soil horizons of the three herbaceous communities. Soil sampling was done using the same design as for SWC. At each sampling date, soil inorganic N contents were measured on the 45 samples (9 sampling points  $\times$  1 horizon for SG, 9 sampling points  $\times$  2 horizon for TG and 9 sampling points  $\times$  2 horizon for EG). Monthly *in situ* N transformations were measured using

the buried-bag method (Hart et al. 1994). For logistical issues, measurements of N transformations were only made on 15 soil samples per sampling date (3 sampling points  $\times$  1 horizon for SG, 3 sampling points  $\times$  2 horizon for TG and 3 sampling points  $\times$  2 horizon for EG). Stones, plant roots and earthworms were removed from soil samples, before placing them in polyethylene bags (0.5 mm thick), preventing water movement while allowing air exchange. Soil disturbance by sieving or hand sorting may alter soil physical properties and thereby influence N cycling by increasing the rates of ammonification and nitrification (Drazkiewicz 1996a, b). However, N transformations were measured on disturbed soils because root biomass and earthworm density can be very high in chalk soils (Decaëns et al. 1997; Jamieson et al. 1998), which may affect more the N dynamics (Jamieson et al. 1998; Potthoff et al. 2001). Bags containing 250 g of soil were hermetically closed and put back in the soil for 28 days, before being dug out for analyses. All soils (45 samples for inorganic N contents + 15 samples for N transformations) were stored at 4 °C and sieved at 2 mm. Inorganic N was extracted within 24 h following sampling.  $\text{NH}_4^+$ -N was extracted by shaking 20 g of fresh soil with 100 mL of  $\text{K}_2\text{SO}_4$  (0.5 M) for 1 h and  $\text{NO}_3^-$ -N was extracted by shaking 20 g of fresh soil with 100 mL of deionised water (Smits et al. 2010). Extracts were filtered through Whatman 42 filters, rinsed with  $\text{K}_2\text{SO}_4$  or deionised water and analysed using a continuous flow analyser AutoAnalyser (AA3, BRAN + LUEBBE, Norderstedt, Germany).

### Calculations and statistical analysis

$\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N amounts were converted to  $\text{g N m}^{-2}$  by taking into account the soil bulk density and horizon thickness. Net N-mineralization and nitrification were expressed in  $\text{mg N m}^{-2} \text{d}^{-1}$ . They were calculated for the incubation period by the following equation (Robertson et al. 1999):

$$\text{Net N - mineralization} = [(\text{NO}_3^- - N_f + \text{NH}_4^+ - N_f) - (\text{NO}_3^- - N_0 + \text{NH}_4^+ - N_0)]/T_d \quad (1)$$

$$\text{Net N - nitrification} = (\text{NO}_3^- - N_f - \text{NO}_3^- - N_0)/T_d \quad (2)$$

where (i) the subscripts  $0$  and  $f$  indicate the content before and after *in situ* incubation, respectively, and (ii)  $T_d$  is the incubation time, in days. A negative value indicates microbial net immobilization (Hart et al. 1994).

All statistical analyses were done using the R 2.12.0 software package (R Development Core Team 2010) and statistical significance was set at  $P < 0.05$ . Data normality and homogeneity of variance were tested with Wilk-Shapiro and Bartlett tests, respectively. When necessary, a log transformation was applied. A two-way ANOVA with herbaceous community and season as the main factors was performed for the horizons and soil variables (SWC,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, net N-mineralization and nitrification) over the study year. One-way ANOVA was used to detect differences in soil variables between herbaceous communities for the A horizon. When the ANOVA was significant, Tukey HSD post hoc tests were used to detect significant differences between means. Student's *t*-test was used to test for differences between TG and EG for the same soil variables for the AC horizon. Relationships between SWC and soil N variables ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N, net N-mineralization and nitrification) were also tested using Pearson correlation analysis.

## Results

### Variations and seasonal patterns in soil water properties

Soil water retention curves and mean available water amounts in the three plant communities are presented in Fig. 2a and b. The shape of the retention curve obtained in SG differed from those in TG and EG. Water retention capacity in the A horizon was higher in SG than in EG ( $F=8.29$ ,  $P=0.001$ ) and permanent wilting point was higher in SG and lower in TG and EG ( $F=8.15$ ,  $P=0.002$ ). The amount of available water for plants in the A horizon was significantly lower in SG than in TG and EG ( $F=16.74$ ,  $P < 0.001$ ; Fig. 2b). The total amount of available water for plants along the soil profile was significantly higher in TG and EG than in SG ( $F=27.21$ ,  $P < 0.001$ ; Fig. 2b).

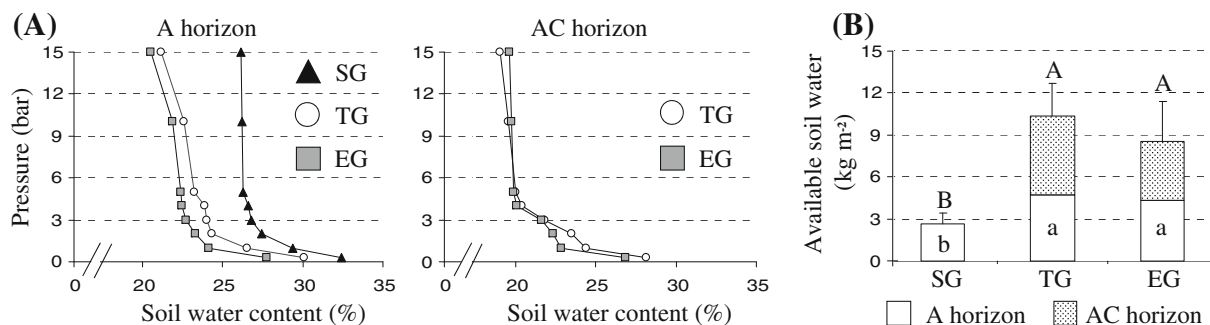
Results from two-way ANOVA demonstrate that community and season as well as their interaction had a significant effect on SWC in the A horizon (Table 2). SWC presented a sharp seasonal dynamic within the three herbaceous communities (Fig. 3). In the A horizon, observed values were significantly higher in SG than in TG and EG in winter (January and February) and autumn (November) ( $F=9.66$ ,  $F=11.23$ ,  $F=6.88$ ,  $P < 0.01$ ). These values were also higher although more variable than in the AC horizon. A significant hot and dry event was observed for about 2 months (August and September) and during this period SWC decreased below 20 %. Afterwards, values strongly increased in October following rain events and gradually rose during the winter to culminate above 35 %.

Permanent wilting point of plant species measured according to the biological method was lower than permanent wilting point of soils for all herbaceous communities and horizons (Fig. 3). It was also lower than the lowest summer values of SWC. Minimal values were measured for *S. caerulea* and *H. nummularium* with 3.13 and 3.70 %, respectively. Intermediary values were found for *T. chamaedrys*, *H. comosa* and *B. pinnatum* with 4.56, 5.44 and 6.05 %, respectively. Maximum values were obtained for *F. lemanii* and *C. flacca* with 7.23 and 6.87 %, respectively.

Soil water content and water loss rate after a rainfall event are presented in Fig. 4. No significant difference was detected between the three communities for soil water content one day after rainfall ( $F=0.43$ ,  $P=0.654$ ). However, significant differences were found for soil water loss rate with a faster decrease in SG than in TG and EG ( $F=42.78$ ,  $P < 0.001$ ).

### Soil N dynamics

$\text{NH}_4^+$ -N content in the A horizon showed a significant seasonal variability over the study period (Table 2 and Fig. 5a). Values were low in spring (except in March), increased in summer and decreased again in autumn and winter. The three herbaceous communities presented similar patterns and significant differences were only found in June, with lower values observed in SG compared to TG and EG ( $F=12.42$ ,  $P < 0.001$ ).  $\text{NH}_4^+$ -N represented the main proportion of the extractable inorganic N in summer, especially during the



**Fig. 2** Soil water retention curves for the A and the AC horizons (a) and available soil water (mean values  $\pm$  SE,  $n=9$ ) in the three herbaceous communities (b). SG Short Grasslands, TG Tall Grasslands, EG Encroached Grasslands. Different letters

indicate significant differences between herbaceous communities at the probability level of 0.05 (Tukey HSD; small letters for the A horizon and capital letters for the whole soil profile)

driest months (August and September), during which it represented 50 to 85 % of the total inorganic N. Similar patterns of variations in  $\text{NO}_3^-$ -N contents were observed in the three herbaceous communities (Fig. 5a), with the higher values observed in autumn and winter while a significant decrease occurred in spring and summer.  $\text{NO}_3^-$ -N was the largest component of the extractable inorganic N in winter, spring and autumn, representing between 60 and 70 % of soil inorganic N.

Patterns of  $\text{NH}_4^+$ -N in the AC horizon were similar in TG and EG (Fig. 5b). Two-way ANOVA showed significant seasonal and community effects (Table 2). The lowest values were found in spring (April and May) whereas the highest were obtained in late summer (September). There were marked significant differences between communities in January, April, and October, with consistently higher values in TG than in EG ( $t=3.24$ ,  $t=3.97$ ,  $t=4.90$ ,  $P<0.01$ , respectively). The amounts in  $\text{NO}_3^-$ -N varied similarly between communities and significant differences were only observed in March with higher content in EG than in TG ( $t=-2.47$ ,  $P=0.025$ ). Values were at a minimum in spring (March and May) and maximum in winter (January and December).

In the three herbaceous communities, net N-mineralization fluctuated significantly over the study period (Table 2). In the A horizon, net N-mineralization peaked in May and was lowest in September for all herbaceous communities (Fig. 6a). Maximum mineralization was observed in EG while lower values were found in SG and TG. The seasonal dynamic of net N-mineralization was also quite specific in SG when

compared to TG and EG. For instance, N immobilization was high in SG ( $-11.2 \text{ mg N m}^{-2} \text{ d}^{-1}$ ) and comparatively low in EG ( $-4.8 \text{ mg N m}^{-2} \text{ d}^{-1}$ ) in September. Significant differences were also found in July with lower mineralization in SG than in TG ( $F=22.59$ ,  $P<0.001$ ). On the other hand, net N-mineralization was higher in SG than in TG and EG in autumn (October and November). In the AC horizon, patterns of variations were more or less similar in all communities, with the highest values of net N-mineralization in May and N immobilization in September and December (Fig. 6b). Cumulative N-mineralization was significantly higher in EG ( $8.7 \text{ g N m}^{-2}$ ) than in SG ( $4.5 \text{ g N m}^{-2}$ ) in spring (April, May, and June) along the soil profile ( $F=8.33$ ,  $P=0.009$ ). No significant difference was established between TG ( $7.1 \text{ g N m}^{-2}$ ) and the other communities.

Patterns of net N-nitrification were comparable to those of N-mineralization and varied significantly over time (Table 2 and Fig. 6b). In the A horizon, similar dynamics were observed for the three communities, with highest values obtained in May in EG and lower values in TG and SG. N immobilization was observed in December for all herbaceous communities ( $-3.9$  to  $-4.8 \text{ mg N m}^{-2} \text{ d}^{-1}$ ). Net N-nitrification was significantly higher in SG than in TG in March ( $F=5.78$ ,  $P=0.032$ ) and significantly lower in SG than in EG in October ( $F=12.38$ ,  $P=0.005$ ). In the AC horizon, maximum nitrification was observed in May and N immobilization in December. There were only significant differences between communities in May with higher values in EG than in TG ( $t=-3.29$ ,  $P=0.022$ ).

In the A horizon, significant negative correlations were found between SWC and  $\text{NH}_4^+$ -N for all



**Table 2** Results of the two-way ANOVA for the soil variables (soil water content,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , net N-mineralization, and net N-nitrification) in the two horizons (A and AC). Significant correlations are shown in bold (\*\*\*)  $P < 0.001$ , \*\*  $P < 0.01$

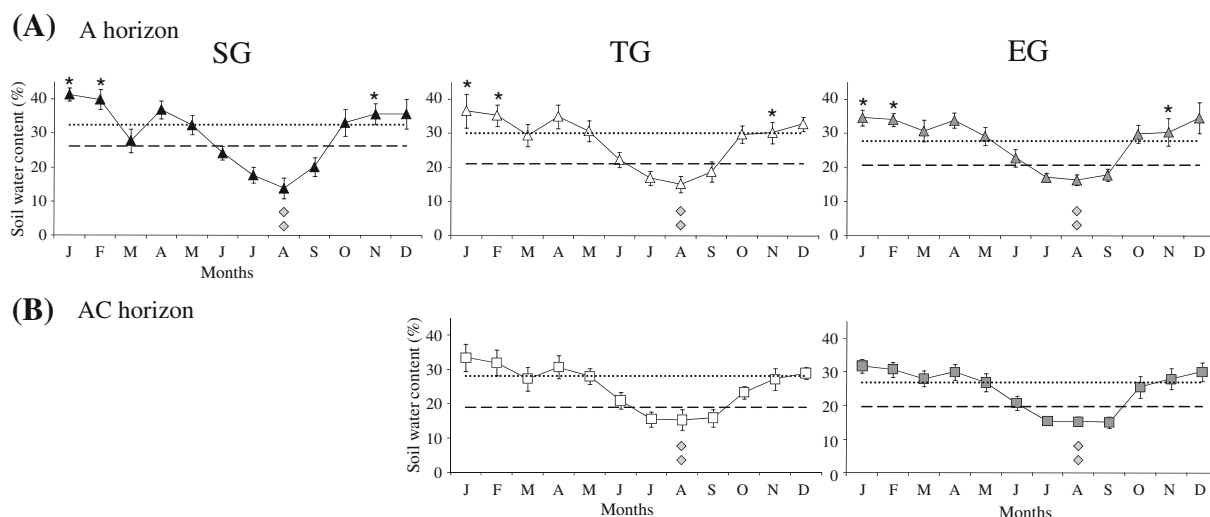
Factor	A horizon					AC horizon						
	df	Soil water content	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	net N-mineralization	net N-nitrification	df	Soil water content	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	net N-mineralization	net N-nitrification
Herbaceous community	2	<b>20.14</b> ***	0.58	<b>6.82</b> **	1.13	<b>4.98</b> **	1	0.04	<b>47.39</b> ***	0.21	3.34	0.03
Season	11	<b>193.30</b> ***	<b>44.13</b> ***	<b>19.81</b> ***	<b>36.12</b> ***	<b>24.43</b> ***	11	<b>107.91</b> ***	<b>20.94</b> ***	<b>10.30</b> ***	<b>70.30</b> ***	<b>32.32</b> ***
Herbaceous community $\times$ Season	22	<b>2.65</b> ***	1.09	0.64	<b>4.52</b> ***	<b>2.59</b> ***	11	0.78	1.35	1.05	<b>4.24</b> ***	<b>3.29</b> ***

herbaceous communities (Table 3). Conversely, positive correlations were observed between SWC and  $\text{NO}_3^-\text{-N}$  in SG ( $r=0.64$ ,  $P=0.02$ ), TG ( $r=0.60$ ,  $P=0.038$ ) and EG ( $r=0.65$ ,  $P=0.02$ ). In the AC horizon, SWC was positively correlated with  $\text{NO}_3^-\text{-N}$  ( $r=0.64$ ,  $P=0.03$ ) and negatively with  $\text{NH}_4^+\text{-N}$  ( $r=-0.62$ ,  $P=0.024$ ) in EG only. There was no significant relationship between SWC and net N-mineralization or nitrification.

## Discussion

Variations and seasonal patterns in soil water properties

In this study, we investigated variations in soil water properties and provided evidence for substantial differences between communities. We detected significant differences in permanent wilting point of soils between SG and the two other communities and found that soil water retention capacity in the A horizon was higher in SG than in EG. Such differences may result from the ability of soils to retain water differently depending on their texture and organic matter content (Lavelle and Spain 2001). In our case, clay content in the A horizon was similar between communities, which indicates that the differences we observed may be related to higher amount of organic carbon in SG (Table 1,  $8.24 \text{ kg m}^{-2}$  soil organic matter in SG ( $4.79 \times 1.72$ ) versus  $5.97$  and  $6.35 \text{ kg m}^{-2}$  soil organic matter in TG ( $3.47 \times 1.72$ ) and EG ( $3.69 \times 1.72$ ), respectively). As expected in our first hypothesis, we found that available soil water for plants was significantly higher in TG and EG than in SG. This is in accordance with Casas and Ninot (2007) who found similar trends in a comparative study between a xerophilous pasture and mesoxerophilous grassland and emphasized that differences in soil properties may have an incidence on soil water storage. In the calcareous grasslands of the Burren, Jeffrey (2003) observed a maximum water storage of  $5.8 \text{ kg m}^{-2}$ , which is comparable to what we found in the A horizon of the three communities studied. We also found that available soil water for plants was at a minimum in SG when considering the whole soil profile. This low water storage is expected to favour xerophilous plants (*S. caerulea*, *H. nummularium*) in SG because these deep-rooted species (>50 cm) have the capacity to penetrate cracks in the



**Fig. 3** Monthly variations (mean values $\pm$ SE,  $n=9$ ) of soil water content (%) in the three herbaceous communities for the A horizon (a) and for the AC horizon (b) over the year 2009. SG Short Grasslands, TG Tall Grasslands, EG Encroached Grasslands. Dashed lines indicate soil permanent wilting point and dotted lines indicate soil water retention capacity. Grey diamonds show permanent wilting point of plant species present in the three

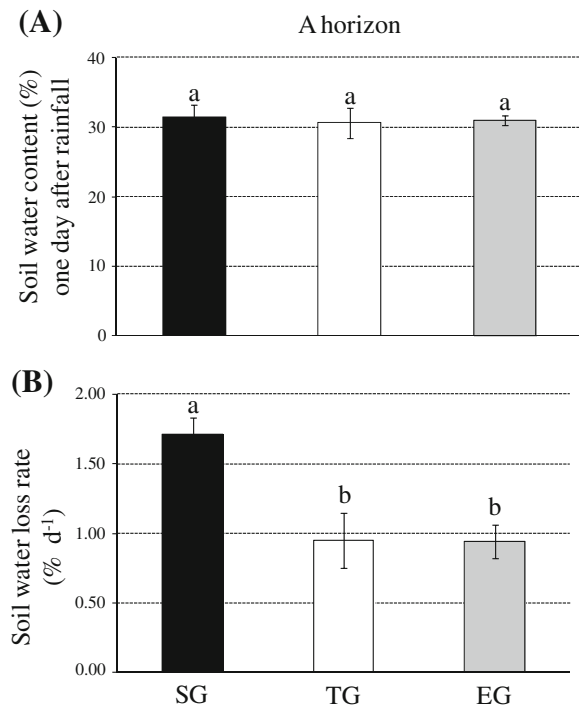
communities. The displayed values correspond to the highest (*Festuca lemanii*) and the lowest species (*Sesleria caerulea*). Permanent wilting point of species is positioned below the driest month (August). \* indicates significant differences between herbaceous communities at the probability level of 0.05 (Tukey HSD and Student's *t*-test)

chalk subsoil and get water, which gives them an advantage in times of drought. In contrast, mesophilous species (*F. lemanii*, *C. flacca*) having shallow root system (<10 cm) can't exploit water in rock crevices and therefore have difficulty surviving a severe water deficit (Salisbury 1952).

Temporal patterns of SWC presented a similar seasonal dynamic between communities, suggesting that it was closely related to climatic variations. This is congruent with the results of Leuschner (1989) who found significant seasonal variations of SWC in calcareous grasslands as a consequence of temporal changes in precipitation, temperature and evapotranspiration. It should also be noted that SWC was higher in SG at some periods of the year (January, February, and November), which may result from differences in environmental conditions between communities (bare soil, amounts of biomass and litter). Moreover, SWC was below permanent wilting point during four months in SG whereas it was only three months in the other herbaceous communities. This is consistent with the drought period that vegetation endured from August to September which was unusually intense compared with a normal year (about 80 % less precipitation). In addition to a low water storage, the longer

water deficit period observed in SG may represent an additional constraint that may cause the death of mesophilous while promoting xerophilous species. This additional month below the soil permanent wilting point may have seriously affected the vegetation of SG because it occurred at a time of year (June) when some plant species were actively growing. For some perennial species (*B. pinnatum*, *T. chamaedrys*), that usually flowered later (July, August), this early water deficit may have significantly reduced their growth and thus their reproductive success. This has been already suggested by Anderson (1927) who observed similar tendencies in British calcareous grasslands and pointed out that an early drought could influence more strongly plant development than a longer drought occurring in late summer when species have completed their seed production.

As expected in our second hypothesis, differences in soil water regime occurred between communities. Similar results have been found by Leuschner (1989) who showed variations in the water dynamics between dry grassland communities with, in particular, a greater water shortage in *Xerobromion* communities in comparison with *Mesobromion* communities. In our study, it was possible to detect the differences in soil



**Fig. 4** Soil water content (%; mean values $\pm$ SE,  $n=6$ ) one day after rainfall (**a**) and soil water loss rate (% d<sup>-1</sup>, mean values $\pm$ SE,  $n=6$ ) between two rainfall events (**b**) in the A horizon for the three herbaceous communities. Measurements were made during a period of 10 days without rain (May 26th–June 7th, 2009). *SG* Short Grasslands, *TG* Tall Grasslands, *EG* Encroached Grasslands. Different letters indicate significant differences at the probability level of 0.05 (Tukey HSD)

water regimes among communities because 2009 was a special year with a severe dry event in the summer. However, these differences would probably have been less pronounced in wet conditions because under normal conditions, precipitation in the lower Seine valley is evenly distributed throughout summer. This leads us to believe that the drought of 2009 was a punctual drought corresponding to a strong disturbance. Moreover, it can be questioned if the occurrence of such climatic conditions may have affected plant survival and species abundance. According to Hopkins (1978), extreme drought may induce important but temporary fluctuations in species abundance and floristic composition in calcareous grasslands. Indeed, only a water stress occurring recurrently can affect the structure of herbaceous communities and their floristic composition in the long term (Alard and Balent 2007). However, our results did not allow testing this idea in a relevant way, as the membrane press method, which is

suitable to measuring permanent wilting point for mesophilous species, may not be efficient to assess this properties for xerophilous plants that are usually able to absorb water below this threshold (Gobat et al. 2003; Casas and Ninot 2007).

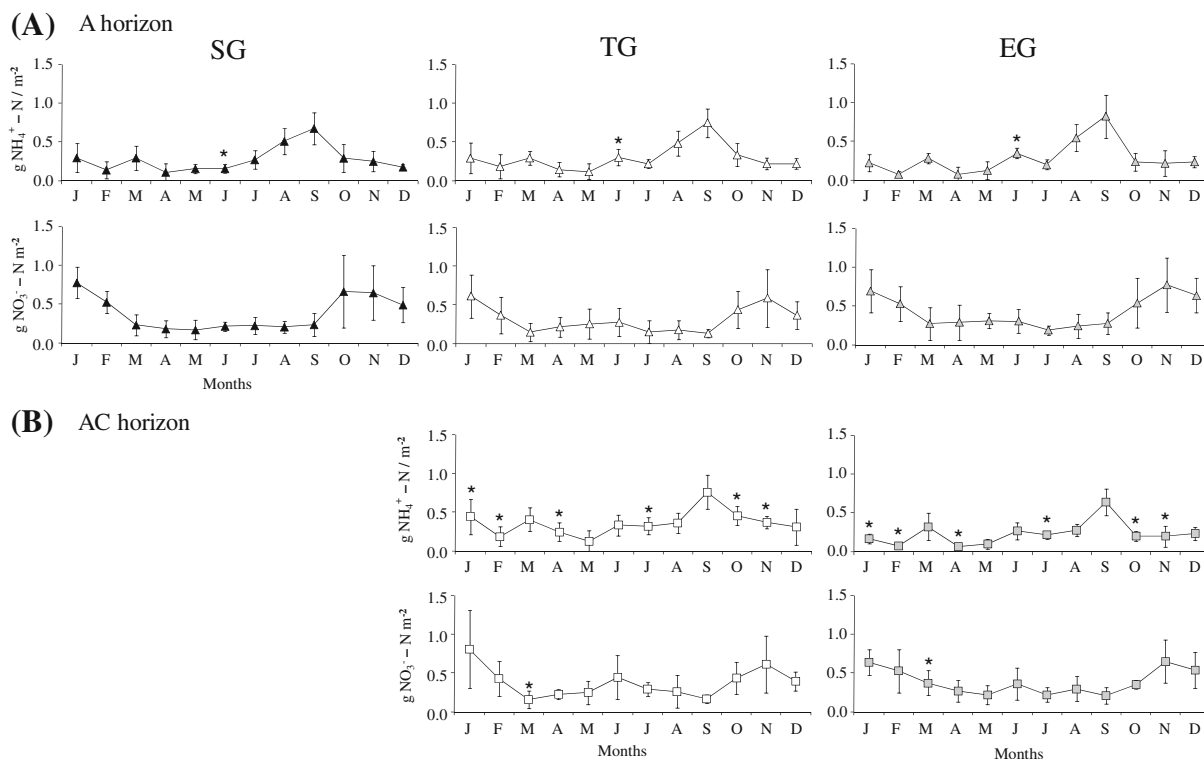
Permanent wilting points of plants measured according to the biological method were comparable to those measured by Anderson (1927) and consistent with data proposed by Gobat et al. (2003) for xerophilous and mesoxerophilous species. All values were below the lowest SWC for all herbaceous communities and horizons, indicating that species can survive a strong water deficit. This is in accordance with Rodriguez-Iturbe and Porporato (2004) who suggested that the permanent wilting point of plants living in dry environments is generally lower than the permanent wilting point of soils. Furthermore, the values of permanent wilting point of plants were close to those proposed by Locket (1946) for the wilting point of chalk (4–5 %). Therefore, this suggests that only a drought of very high intensity could threaten the survival of these species.

SWC variations following a rainfall event also showed that soil dried more rapidly in SG than in TG and EG. The soil water loss was higher in SG because soils were thin and abiotic constraints were restricting. In addition, soil water evaporation may be higher under a low vegetation cover. In contrast, TG and EG were characterized by higher amount of litter and deeper soils, which could buffer variations in SWC and reduce soil water evaporation.

#### Soil N dynamics

Contrary to our third hypothesis, seasonal variation of soil inorganic N presented few significant differences between herbaceous communities, both for the A and the AC horizons. NO<sub>3</sub><sup>-</sup>-N was the predominant form of inorganic N (except in summer) and estimated amounts were consistent with those found by Leuschner (1989) for calcareous soils. Temporal patterns of inorganic N contents were also very similar between herbaceous communities and for the two horizons. However, some clear variations were observed during the studied period and changes in N inorganic contents occurred according to season and water availability.

We found that NH<sub>4</sub><sup>+</sup>-N levels decreased in spring and increased during drought period in each horizon for all herbaceous communities. This is in accordance



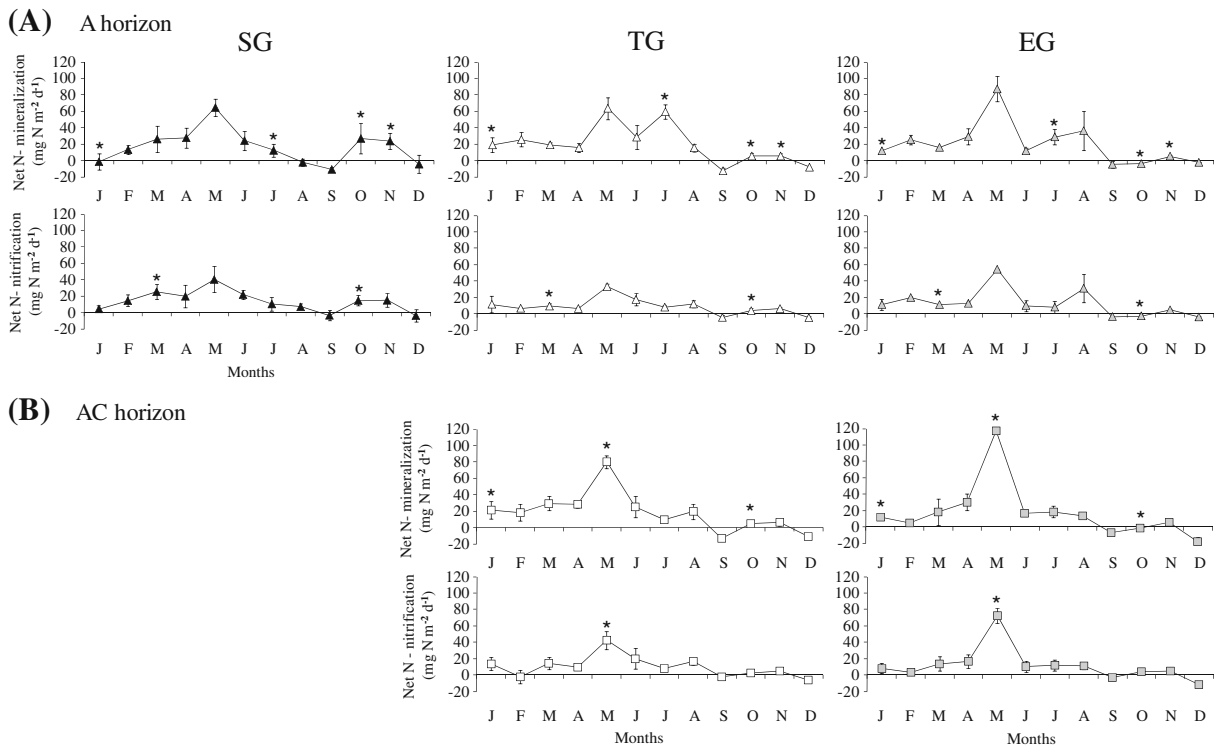
**Fig. 5** Monthly variations (mean values $\pm$ SE,  $n=9$ ) of soil  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  levels over the year 2009 in the three herbaceous communities for the A horizon (a) and for the AC horizon (b). SG Short Grasslands, TG Tall Grasslands, EG

Encroached Grasslands. \* indicates significant differences between herbaceous communities at the probability level of 0.05 (Tukey HSD and Student's  $t$ -test)

with Giese et al. (2011) and Ellenberg (1977) who found that  $\text{NH}_4^+\text{-N}$  content was higher in summer when SWC decreased. One explanation for this pattern is that  $\text{NH}_4^+\text{-N}$  content is mostly driven by plant uptake which fluctuates depending on the season (Jamieson et al. 1998). Alternatively, soil organic matter content, which can increase during dry periods due to senescence and loss of aboveground and root biomass, may also trigger an increase in  $\text{NH}_4^+\text{-N}$  production. This was described by Jamieson et al. (1998) who observed an increase in soil Corg during summer, which they interpreted as the effect of large inputs of fresh plant debris. As root biomass is the dominant living plant component in calcareous grasslands, dead root decomposition probably represents a major source of N release at this time of year. Finally,  $\text{NH}_4^+\text{-N}$  release may also depend on microbial activity, which could be inhibited at low soil water potentials (Stark and Firestone 1995). In particular, nitrifiers could be more sensitive than ammonifiers below permanent

wilting point (Unkovich et al. 1998). Moreover, soil drying may enhance the amount of  $\text{NH}_4^+\text{-N}$  liberated from soil organic matter and may provide a suitable substrate for nitrifying bacteria once their activity starts again (Appel 1998).

As hypothesized for  $\text{NH}_4^+\text{-N}$ , the temporal variations of  $\text{NO}_3^-\text{-N}$  content can be mainly related to variations in plant uptake between seasons (Jamieson et al. 1998). This plant N uptake is closely related to mycorrhizal fungi which play a significant role on  $\text{NO}_3^-\text{-N}$  uptake under water-stressed conditions (Tobar et al. 1994). Similarly, we can assume that  $\text{NO}_3^-\text{-N}$  content is related to changes in microbial activity depending on climatic conditions. In calcareous soils, most bacterial communities can survive in a state of strongly reduced physiological activity during dry periods (Potthoff et al. 2001). It is thus unlikely that  $\text{NO}_3^-\text{-N}$  peaks observed in autumn result from microbial biomass mineralization by bacterial communities that survived in desiccated soil. Another possible



**Fig. 6** Monthly variations (mean values±SE, *n*=3) of net N-mineralization and nitrification over the year 2009 in the three herbaceous communities for the A horizon (a) and for the AC horizon (b). *SG* Short Grasslands, *TG* Tall Grasslands, *EG*

Encroached Grasslands. \* indicates significant differences between herbaceous communities at the probability level of 0.05 (Tukey HSD and Student’s *t*-test)

explanation is that a summer drought followed by a rainy autumn may favour NO<sub>3</sub><sup>-</sup>-N production from the degradation of non-biomass organic N (Appel 1998). Leaching of NO<sub>3</sub><sup>-</sup>-N may be significant during this period but autumnal rains can also trigger the renewal of earthworm activity.

Macrofauna influence litter decomposition, enhance organic matter decomposition and cause in turn a significant release of inorganic N in the soil (Decaëns et al. 1999; Milcu et al. 2008).

Net N-mineralization and nitrification patterns shared similar seasonal variations. Maximum N-mineralization

**Table 3** Pearson’s linear correlations (*r* coefficient) between soil water content and soil N variables (contents of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, net N-mineralization and net N-nitrification) in the three herbaceous communities for the two horizons (A and

AC). *SG* short grasslands, *TG* tall grasslands, *EG* encroached grasslands. Significant correlations are shown in bold (\**P*<0.05, *n*=12)

	Soil water content (%)				
	A horizon			AC horizon	
	SG	TG	EG	TG	EG
NH <sub>4</sub> <sup>+</sup> -N content (g N m <sup>-2</sup> )	<b>-0.62 *</b>	<b>-0.61 *</b>	<b>-0.69*</b>	-0.47	<b>-0.62*</b>
NO <sub>3</sub> <sup>-</sup> -N content (g N m <sup>-2</sup> )	<b>0.65*</b>	<b>0.60*</b>	<b>0.65*</b>	<b>0.460.64*</b>	
net N-mineralization (mg N m <sup>-2</sup> d <sup>-1</sup> )	0.22	-0.11	-0.06	0.29	0.09
net N-nitrification (mg N m <sup>-2</sup> d <sup>-1</sup> )	0.13	0.02	-0.05	0.02	0.06



in the A horizon ( $63\text{--}87 \text{ mg N m}^{-2} \text{ d}^{-1}$ ) and maximum net N-nitrification ( $33\text{--}54 \text{ mg N m}^{-2} \text{ d}^{-1}$ ) were comparable with data reported elsewhere for other calcareous grasslands (Unkovich et al. 1998). Results were also consistent with those of Leuschner (1989), who found that N transformations principally occurred in May in this ecosystem. Maximum N transformations values were observed at this time of year because SWC and temperature were optimal for microbial activity (Jamieson et al. 1999). For the two horizons, few significant differences in N transformations were found between herbaceous communities over the study period. However, the accumulation of mineralized N throughout the soil profile is higher in EG than in SG in spring, which can affect plant growth at a time when N demand is usually high. In addition, these results are consistent with the differences observed in biomass and potential N-mineralization rate between the two communities (Table 1).

The decrease of N transformations observed during summer, especially in the case of SG, is probably related to the high temperatures and the low SWC (less than 15 %) that occurred during this period (Taylor et al. 1982; Leuschner 1989). It was not related according to our results to a deficit in  $\text{NH}_4^+\text{-N}$  or  $\text{NO}_3^-\text{-N}$ , but it could result from severe microbial activity decrease once SWC falls below the soil permanent wilting point (Ford et al. 2007). N immobilisation was high in late summer (August, September) in SG, suggesting that microbial communities and plants may compete for N. Possibly, this interaction may affect some dominant species such as *T. chamaedrys* whose flowering and seed production occurs in late summer. N transformations remained high during dry months (July and August) in TG and EG compared with SG. These transformations were probably sustained by the buffering of litter that induced a more favourable microclimate for microbial activity (Schimel and Parton 1986). Alternatively, shifts in ammonia-oxidising communities may be suspected since floristic composition differs between herbaceous communities (Chabrerie et al. 2003). Small differences in microbial composition would thus explain differences in N transformations in those grasslands stands (Kowalchuk et al. 2000).

N transformations also occur in autumn (October, November) in SG in spite of the presumably low microbial activity induced by cold temperatures (Jamieson et al. 1999). Similar results have been reported by Davy and Taylor (1974a) who detected

active nitrification in chalk soils during laboratory incubations at  $2^\circ\text{C}$ . In the same study, these authors also found a high level of N-mineralization in November and noticed that this peak occurred after a very dry period. Such a peak may result from rewetting after summertime soil desiccation, which could have stimulated microbial communities and N mineralization rate. Significant amounts of inorganic N could be thus released during this season, which can enhance nitrogen lost through  $\text{NO}_3^-\text{-N}$  leaching due to the poor development of the vegetation and the intensity of precipitation at this time of year (Jamieson et al. 1998). Conversely, it could be noticed that N immobilization occurred in October in EG, which may limit N leaching losses in this community.

Soil N transformations in grassland ecosystems vary from year to year due to variability in climatic conditions such as temperature or rainfall (Davy and Taylor 1974a; Giese et al. 2011). In our study, the results were collected during a dry year during which water availability was a key limiting factor for N transformations in summer. However, it is likely that soil N dynamics would have been different in wetter climatic conditions, as this was already mentioned by Ellenberg (1977) in a study comparing net N-mineralization in a rendzina under limestone-beechwood during a dry and a relatively wet year. Moreover, this author pointed out that the annual amount of mineralized N in chalk soils is clearly higher under beechwoods than under dry grasslands, which suggests that water supply is not the only factor that limits N transformations in grassland soils. Indeed, high surface temperatures and solar radiation may create a hot and dry microclimate causing rapid soil drying and thus depressing SWC in grasslands (Morecroft et al. 1998). Similarly the quantity, quality and accessibility of soil organic matter may be considered as a major cause keeping down N dynamics in those soils (Maire et al. 1999). Furthermore, the form and quality of litter falling on the soil and the composition and abundance of decomposers and their activities may be of great importance for explaining the lower soil N transformations in calcareous grasslands (Milcu et al. 2008).

## Concluding remarks

A substantial finding in our study is that SG presented a specific soil functioning compared to other communities. This highlights the problem of the chronological

link between communities as our results suggest that different herbaceous communities may represent stages of different soil-driven successional dynamics rather than different stages of a single successional gradient. Accordingly, our results suggest some caution in the use of synchronic approaches (i.e. space for time substitution) in studies of vegetation dynamics in calcareous hillsides. These approaches should consider soil conditions in the elaboration of sampling design to avoid misleading results due to the confounding effect of soil factors in between-stages comparisons. This also emphasizes the need for considering secondary succession not as an unidirectional process but rather as a range of processes characterized by different successional trajectories or different rates of vegetation changes. From a conservation management perspective, this conclusion is important because classical management options that intent to promote the conversion of tall grasslands into species-rich short grasslands through intensive grazing may be impaired if local soil conditions drive the system toward an unintended trajectory. Therefore, any attempt to induce the reversal of succession should consider local soil conditions to increase the probability of success in the reestablishment of species-rich short grasslands.

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