

Distribution and effects of tree leaf litter on vegetation composition and biomass in a forest–grassland ecotone

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Abstract

Aims

After abandonment of grasslands, secondary succession leads to the invasion by woody species. This process begins with the accumulation of tree litter in the forest–grassland ecotone. Our objectives were to determine the relationships between litter amounts and vegetation composition and cover along natural forest–grassland ecotones and to experimentally study the initial effects of tree litter accumulation on grassland vegetation and on microsite conditions.

Methods

We established 11 transects varying from 12 to 15 m in length in different forest–grassland ecotones in the Lahn-Dill highlands, Germany, and measured the mass and cover of tree litter and the cover and composition of vegetation at five sequential positions along each transect by using 1 m² plots with five replications. In a field experiment, we established plots subjected to different litter amounts (0, 200 and 600 g m⁻²) and evaluated changes in grassland vegetation, soil temperature and soil nutrient availability below the litter layer.

Important Findings

Tree litter amounts decrease from 650 to 65 g m⁻² across the forest–grassland ecotone. Vegetation changed from shrubs and annual

species (adapted to more stressful conditions) in the forests edge to grasses, rosettes and hemirosette species (with higher competitive abilities) in the grassland. These anthropogenic forest–grassland ecotones showed abrupt edges, and the two adjacent ecosystems were characterized by different species pools and functional groups. In the field experiment, the presence of a litter layer reduced vegetation biomass and cover; the species richness was only reduced in the treatment with high litter (600 g m⁻²). Additionally, adding litter on top of vegetation also reduced thermal amplitude and the number of frost days, while increasing the availability of some nutrients, such as nitrogen and aluminium, the latter being an indicator of soil acidification. Adding a tree litter layer of 600 g m⁻² in grassland areas had strong effects on the composition and diversity of grassland vegetation by reducing the cover of several key grassland species. In, or near, forest edges, litter accumulation rapidly changes established vegetation, microsite conditions and soil nutrients.

Keywords: forest edge, land abandonment, land use change, litter, oak (*Quercus robur*), semi-natural grassland, tree invasion

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INTRODUCTION

Semi-natural grasslands in Europe, which originated through man-made forest clearings in prehistoric and historic times with the aim to create agricultural areas for pasturing or hay making (Dierschke and Briemle 2002; Myster 2012), can only

be maintained through continuous anthropogenic use (Veen *et al.* 2009). These are among the most diverse ecosystems in Europe and thus have a high conservation value (Critchley *et al.* 2004; Hodgson *et al.* 2005). However, in many cases these grasslands were abandoned and mowing and grazing regimes ceased completely (Moog *et al.* 2002; Quéfier *et al.* 2007). This

leads to a process of secondary succession where grasslands are invaded by woody species (Didier 2001; Myster 1994; Steven 1991). Currently, increased forest productivity and litter fall as a consequence of elevated CO₂ levels (DeLucia *et al.* 1999; Finzi *et al.* 2001; Zak *et al.* 2003) may accelerate this process.

The ecotones between forest and grasslands are more vulnerable to change by colonization of forest species (Didier 2001; Myster 2012) with subsequent loss of species diversity (Dierschke 1974; Pärtel *et al.* 2005; Sydes and Grime 1981a). The advance of forest edge begins with the accumulation of tree litter in nearby grassland areas, which suppresses grassland herbs and may even lead to their disappearance (Donath and Eckstein 2008). Deciduous tree litter acts as a strong selection agent because it may modify the growth environment for seeds, seedlings and adult plants (Baltzinger *et al.* 2012; Molofsky *et al.* 2000; Stinchcombe and Schmitt 2006). Grime (2001) suggested that tree leaf litter accumulation in these ecotones may improve woody seedling establishment by reducing the competitive effect of grassland vegetation. These indirect effects of tree leaf litter on competition with grasses may benefit woody species or woodland forbs (Facelli 1994; Sydes and Grime 1981a). Some species, as those with a higher relative growth rate (e.g. erect herbaceous species and shrubs) or with storage organs (e.g. geophytes) seem to be better adapted to penetrate a litter layer. This is especially true for species with robust shoots (Cintra 1997; Grime 2001), since they are capable of penetrating even a thick litter layer. Also rosette species have the ability to place their leaves above the litter layer, displace it and open a gap for the subsequent growth of new leaves (D'Angela *et al.* 1986; Facelli 1988). Other types of vegetation, with weaker leaves and stems, like annual species (Wilson and Zammit 1992), may not be able to withstand conditions generated by a dense litter layer.

However, the response of vegetation depends on the amount of litter present (Eckstein and Donath 2005; Gross 1984; Xiong and Nilsson 1999). While in the presence of a thin layer positive effect may prevail, litter accumulation above certain threshold results in negative effects on vegetation establishment (Jensen and Gutekunst 2003; Quedsted and Eriksson 2006; Violle *et al.* 2006). In a meta-analysis across several ecosystem types, including forests, Xiong and Nilsson (1999) identified this threshold at an amount of ~200 g m⁻² or a layer thickness >1.5 cm, while Loydi *et al.* (2013) reported a tipping point at 500 g m⁻² for grass litter in grassland ecosystems. Fragment size is also an important litter trait, since larger fragments (as whole deciduous tree leaves) may have more negative effects on vegetation than small fragments (Xiong *et al.* 2001).

Xiong and Nilsson (1999) found a strong negative effect of tree litter on seed germination and establishment, but also at the community level litter may reduce species richness and above-ground biomass. Tree litter may present a mechanical barrier to seedling emergence impeding or diminishing emergence (Donath and Eckstein 2008; Facelli and Pickett 1991)

or it may change physical conditions such as soil temperature, moisture regime and light quantity and quality (Holmgren *et al.* 1997). Litter can also act as a seed trap, preventing seeds from reaching the soil (Ruprecht and Szabo 2012), promoting pathogenic fungi (Facelli *et al.* 1999) or increasing seed predation (Reader 1991, but see Myster and Pickett 1993). In all cases, this may reduce germination and lower number of established seedlings. However, different climatic conditions, especially high temperatures and low water availability, may result in positive instead of negative litter effects on seedling establishment (Boeken and Orenstein 2001; Eckstein and Donath 2005; Ruprecht *et al.* 2010). Furthermore, nutrient release during decomposition of litter may increase plant available nutrients (Aerts 1997; Koorem *et al.* 2011), promoting plant growth and relaxing competition.

Despite their high nature conservation value, forest–grassland edges are still subject to rapid decline in quality and quantity through human activities (Fagan *et al.* 1999). In this context, since litter is one of the major factors controlling processes in these ecotones (Myster 2012), it is important to study in detail the spatial distribution and accumulated amount of tree litter in forest–grassland ecotones and evaluate the response of grassland vegetation to the presence of different litter amounts. This will help to predict the response of different herbaceous communities to the advance of the forest edge. To this end, we addressed the following hypotheses:

- (1) The accumulation of tree litter is reduced with distance to forest, and accordingly vegetation composition changes from tolerant to competitive species, with higher growth rate.
- (2) Tree leaf litter accumulation reduces the richness and biomass of grasslands species, and species composition change towards communities dominated by erect growing species and rosette species.
- (3) Tree leaf litter improves microsites conditions by reducing temperature fluctuations and increasing soil nutrients availability.

MATERIALS AND METHODS

Data collection

The study was conducted in two locations in the Lahn-Dill highlands, Hessen, Germany. First, we sampled forest–grassland ecotones at 11 sites near the village Eismroth (50°43'53.19"N, 08°24'53.78"E, ~400 m a.s.l.). Second, we performed a field experiment on a grassland site close to the village Allna (50°46'15.79"N, 08°40'13.79"E, ~217 m a.s.l.), located ~18 km east of Eismroth. Both localities share similar climatic and edaphic conditions; mean annual temperature in the region is 7–8°C and mean annual precipitation ~1000 mm (HLUG 2009).

For the forest–grassland ecotone, we selected 11 sites with similar edaphic conditions, inclination and elevation. Criteria for selection of sites were to (i) cover a forest–grassland ecotone with deciduous forests dominated by *Carpinus betulus* L., *Fagus sylvatica* L. and *Quercus* spp, (ii) be mown 1–2 times

a year and (iii) be maintained without the application of fertilizers. In each site, we established one transect from forest to grassland. Each transect had five sampling positions (P) that were defined in relation to the forest edge; P1: 3 m inside the forest edge, P2: 2 m outside the forest edge, P3: below the tips of the outermost branches of the forest edge trees, P4: 2 m away from P3 and P5: 1 m away from P4. The transect length thus varied between 12 and 15 m. Forest edge was defined as the line of 5 m high trees with interlocking branches and a cover >70% (Mueller-Dombois and Ellenberg 1974). Thus, P1 was situated inside the forest, P2 and P3 in the forest–grassland ecotone and P4 and P5 were located in the grassland area. During November 2010, we measured tree litter cover and dry litter mass, and vegetation cover at each position. Each measurement was taken in 1 m² square plots with five replicates per position per site. The five replicates were averaged for each position in each transect in the 11 sites ($N = 55$). Litter and vegetation cover were visually estimated with 10% cover ranges. Tree litter biomass was collected in the plots, cleaned and oven-dried for 48 h at 60°C. During spring 2011, we established one transect at each site and assessed vegetation cover at the species level in a 1 m² square plot per position. At P1, only understory vegetation was sampled. Cover was visually estimated using a modified Braun-Blanquet scale (van der Maarel 1979). Additionally, we measured percentage of light transmission using a fisheye lens (Soligor DHG 0.19x, Ø 0.37 mm). Total light transmission was calculated with SideLook software version 1.1.01 (Nobis 2005).

For our field experiment, we established 45 plots of 0.5 × 0.5 m² in a systematic square design (9 rows × 5 columns) with a 45-cm buffer area between plots. The experimental area was established in an unfertilized homogenous pasture area near a forest (i.e. between 3 and 8 m from the forest edge), which was mown once a year (mid-June). The experiment started before the beginning of the growing season, when vegetation was still not developed (i.e. vegetation height was ~1 cm). It was carried out for 4 months (March–June) during 2012. During the experimental period, no management was performed in the pasture area. To study the effects of litter, each plot received one of three litter amounts. These were control (no litter), low litter amount (50 g per plot) or high litter amount (150 g per plot). Low and high litter amounts correspond to 200 and 600 g m⁻², respectively. These litter amounts were chosen because they represent the range found along forest edges in the study area (see Results). Leaves of oak (*Quercus robur* L.) were collected in a mixed deciduous woodland during dry weather in the nearby area and air dried for 2 weeks. For each treatment, 15 replicates were established. In three replicates of each treatment, we monitored hourly temperature using temperature data loggers (Tinytag Transit, Gemini Dataloggers Ltd, Chichester, UK) from 1 March 2012 to 25 June 2012. In four replicate plots of each treatment, we estimated soil supply rates for nitrate (NO₃⁻-N), ammonium (NH₄⁺-N), phosphorus (P), potassium (K), sulphur (S),

calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B) and aluminium (Al) using PRSTM-probes (Western Ag Innovations, Saskatoon, Saskatchewan, Canada). PRSTM-probes consist of an ion-exchange membrane that adsorbs ions through electrostatic attraction, simulating a plant root. Two anion probes and two cation probes were buried in the top 5 cm of soil at a 45° angle for two consecutive 4-week periods in May (25 April to 23 May 2012) and June (24 May to 19 June 12). After removal, the PRSTM-probes were washed with deionized water and returned to Western Ag Innovations for analysis of adsorbed ions: NO₃⁻-N and NH₄⁺-N by an automated colourimetry flow injection analysis system and all other ions by inductively coupled argon plasma spectrophotometry (Hangs et al. 2004). In each plot, we visually estimated total litter cover and made a vegetation relevé at the end of the study period. Cover was visually estimated using a modified Braun-Blanquet scale (van der Maarel 1979). Additionally, vegetation biomass was collected 1 cm above the soil surface and oven dried for 48 h at 60°C and weighted. Daily precipitation and daily mean air temperature during the experimental period were taken from the German Weather Service (Deutscher Wetterdienst, <http://www.dwd.de>) from a meteorological station in Cölbe, located ~15 km northeast of Allna.

Statistical analysis

Changes in litter and vegetation cover, biomass, species richness and light transmission were tested using nested analysis of variance (ANOVA) for the forest–grassland ecotone, with transects nested within sites, and one-way ANOVA for the field experiment. In all cases, Tukey honest significant difference test (Tukey HSD) was used as means comparison test ($\alpha = 0.05$). Before analysis, biomass data (litter or vegetation) were square-root transformed, cover percentage (litter or vegetation) and percentage of light transmission were arc-sin transformed, and species richness was log-transformed (natural logarithm) to meet ANOVA assumptions. We performed two separate Non-Metric Dimensional Scaling (NMDS) with the species composition from each plot for the forest–grassland ecotone and the field experiment. Species with <5% frequency (occurrence in <2 plots) were not considered. Species cover data were square-root transformed before analysis. The NMDS was performed with Sørensen-distance measure, a maximum of 500 iterations, 6 dimension, 250 real and randomized runs and a starting configuration by random number. For the final solutions, we considered three dimensions for the forest–grassland ecotone and two dimensions for the field experiment. Ordination of the samples in the NMDS was correlated with cover of different plant functional groups and species with different ecological strategies (McCune and Grace 2002). Data for plant functional groups and strategy were taken from Biolflor v1.1 database (Klotz et al. 2002). Nine plant functional groups were defined according to life form and life cycle (Table 1). Ecological strategy follows the C

Table 1: plant functional groups and number of species per group in the forest–grassland ecotone (F–G) and in the field experiment (FE)

Functional group	Number of species		Characteristic
	F–G	FE	
Annual forbs	8	9	Annual herbaceous dicots
Annual graminoids	3	0	Annual monocots
Erect species	25	4	Perennial dicots with only long internodes
Geophytes	5	1	Species with buds in storing organs within the soil
Hemirosettes	43	11	Species with shoots with either long or short internodes
Perennial graminoids	28	15	Perennial monocots
Rosettes	7	10	Species with only short internodes and basal leaves
Shrubs	7	0	Woody species with basal ramification
Trees	11	0	Species with a main woody stem

(competitive species)-S (stress-tolerant species)-R (ruderal species) strategy types by Grime *et al.* (1988). Additionally, in the field experiment, we analysed changes in the cover of the most frequent species (frequency >50%) with different litter treatments. Percentage cover was analysed with a one-way ANOVA and Tukey HSD was used as means comparison test ($\alpha = 0.05$). Prior to analysis, data were transformed to square-root to meet ANOVA assumptions.

In the field experiment, for analysis of mean daily temperature and nutrient availability we used a repeated-measure ANOVA with linear mixed-effect models. Litter treatment was considered a fixed effect. Time was treated as a within factor (von Ende 1993). For nutrient availability, time was considered a fixed factor and changes in nutrient availability in both studied periods, and their interaction with litter amount, were analysed. In all cases, data were transformed to their natural logarithm before analysis, to meet ANOVA assumptions.

All univariate analyses were performed with Statistica (version 10.0, StatSoft Inc., Tulsa, OK, USA) and multivariate analyses were made using PC-ORD (version 5.33, MjM Software, Gleneden Beach, OR, USA).

RESULTS

Changes in established vegetation

Forest–grassland ecotone

Our results showed that there are significant changes in tree litter cover and litter mass along the forest–grassland transect (P1 → P5) (Figure 1a and c; Table S1, see online

supplementary material), ranging from ~95 to 30% in litter cover and from ~650 to 65 g m⁻² in litter mass across the transects. On the contrary, vegetation cover and species richness significantly increased from forest to grassland (Figure 1b and d). Light transmission significantly declined along the forest–grassland transect (P1 → P5) from 1.5 ± 0.5% (mean ± SE) in P1 to 65 ± 5.1 in P5 (Figure S1, see online supplementary material for details).

NMDS performed with three dimensions showed Kruskal's stress of 18.9, which is significantly smaller than the stress expected by chance (Monte-Carlo test, $P < 0.001$). Final interpretation of the ordination did not change between two and three dimensions although Kruskal's stress was lower with three dimensions. For simplicity, we present and interpret the first two dimensions (Figure 2). Vegetation changes were mainly driven by the replacement of tree species in P1 (in the forest) by hemirosettes, rosettes and perennial grasses in P4 and P5 (Figure 2a; Table S2, see online supplementary material). Positions near the forest edge (P2 and P3) were characterized by higher cover of geophytes and shrubs species (P2) and annual forbs and grasses (P3). Additionally, grassland areas were dominated by competitive species (C, CS and CSR species; Figure 2b), while near the forest edge ruderal species were more common (CR and SR species). Forest areas (P1) were related with stress-tolerant species (S), mostly due to one species, *Galium odoratum* (L.) Scop., an erect hemicryptophyte that only appeared in or near forest areas (P1, P2 and P3; Table S2, see online supplementary material).

Field experiment

Litter amounts showed differential effects on vegetation structure (Figure 3; Table S3, see online supplementary material). Low and high litter amounts resulted in significantly different litter covers (Figure 3a). Both amounts also reduced vegetation cover and biomass (Figure 3b and c). However, species richness only showed a significant decrease at high litter amounts, without differences between control and the low litter amount treatment (Figure 3d).

NMDS performed with two dimensions showed Kruskal's stress of 28.2 (Figure S2, see online supplementary material). This was significantly smaller than the stress expected by chance (Monte-Carlo test, $P < 0.001$), but is still high for this type of analysis (McCune and Grace 2002). No plant functional groups or trends in ecological strategies were found in the NMDS performed, showing that there was no clear pattern in species composition of these grasslands. However, cover of some of the most frequent species showed significant differences with litter cover (Figure 4; Table S4, see online supplementary material). Of the 16 analysed species, six showed significant changes in cover values. These were four important and characteristic perennial grasses of mesic grasslands (*Arrhenatherum elatius*, *Bromus hordeaceus*, *Poa pratensis* and *Trisetum flavescens*; Figure 4c, d, k and o), one rosette species (*Leontodon autumnalis*; Figure 4h) and an annual forb (*Veronica arvensis*; Figure 4p). Five of these species

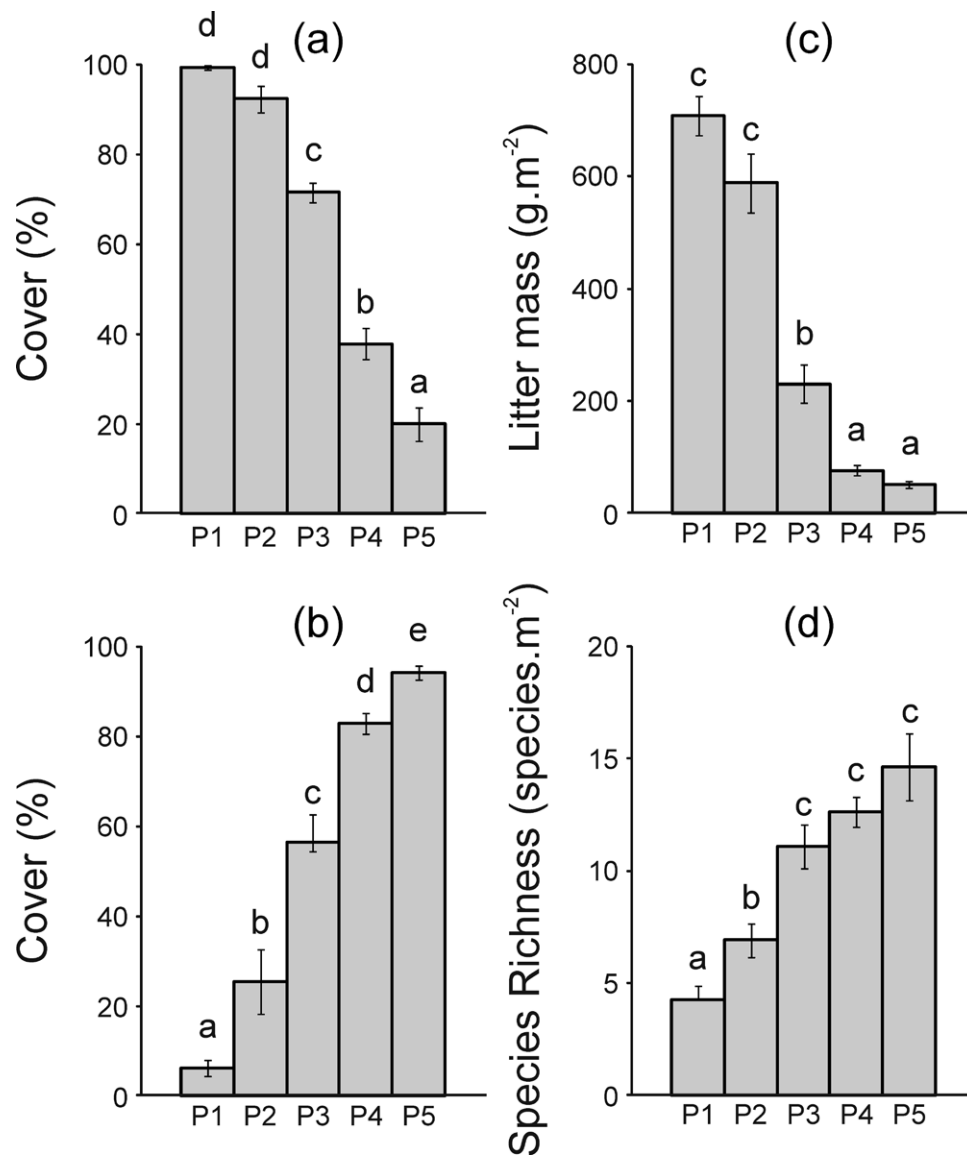


Figure 1: responses of litter cover (a), vegetation cover (b), litter mass (c) and species richness (d) across the ecotone from forest (P1) to grassland (P5) plots. P1 was situated inside the forest, P2 and P3 in the forest–grassland ecotone and P4 and P5 were located in the grassland (see Methods). Bars represent ± 1 SE. Different letters on bars are significantly different mean values (Tukey test at $P < 0.05$).

showed a reduction in cover percentage only with high litter mass, and one grass species (*A. elatius*) showed significant lower covers at low and high litter amounts as compared with controls.

Changes in microsite conditions

Daily temperature showed some differences among control and treatment plots (Figure 5; Table S5, see online supplementary material). The presence of a tree litter layer did not change the mean daily temperature. However, a tree litter layer of 600 g m^{-2} reduced the maximum daily temperature by 2.9°C compared with the control. Also minimum daily temperature has significantly increased by 1.6°C for the same litter amount. Thus, the presence of a high tree litter

layer reduced temperature amplitude (Figure S3, see online supplementary material). Additionally, high litter amounts reduced the number of days with frosts (temperature $\leq 0^\circ\text{C}$, 3 days) compared with control treatment (16 days) or low litter amount treatment (20 days).

The presence of litter had an effect on soil nutrient availability for plants. Nutrients like nitrate, calcium, magnesium, phosphorus and aluminium were higher during the first incubation period than during the second (Figure 6 and Table S6, see online supplementary material). However, only nitrogen (total nitrogen and nitrate) and aluminium showed significant differences among the different litter amounts. Nitrogen increased with high litter amounts in the first period (Figure 6). Aluminium availability was higher with low and

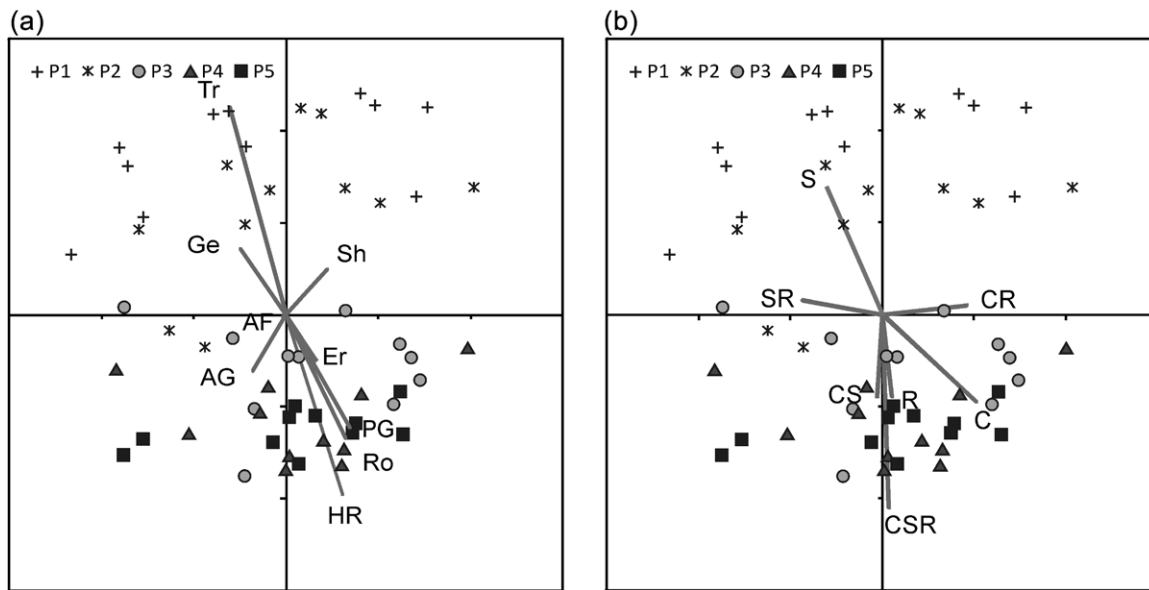


Figure 2: NMDS ordination diagram of vegetation relevés in a forest–grassland ecotone. P1 was situated inside the forest, P2 and P3 in the forest–grassland ecotone and P4 and P5 were located in the grassland (see Methods). Relation between the NMDS ordination and (a) plant functional groups cover (Table 1) and (b) species ecological strategy are shown. AF = annual forbs, AG = annual graminoids, Er = erect species, PG = perennial graminoids, Ge = geophytes, HR = hemirosettes, Ro = rosettes, Sh = shrubs, Tr = trees. Ecological strategies are defined according to Grime *et al.*, 1988. C = competitive species, R = ruderal species, S = stress-tolerant species.

high litter amounts in the first period and with high litter amounts in the second period (Figure 6). The rest of the analysed nutrients showed no changes with litter amount or between the different incubation periods (Table S6, see online supplementary material).

DISCUSSION

The presence of tree litter has clear effects on the composition and diversity of grassland vegetation. Our field experiment suggests that litter accumulation significantly affects the cover of grassland species already in the first growing season and may influence community composition and diversity.

Changes in established vegetation

As expected, litter amount decrease along the studied transects and associated vegetation changed. Grassland areas are characterized by higher abundance of competitive species, such as perennial grasses and rosette and hemirosette species. Perennial grasses and some hemicryptophytic forbs have high competitive ability and thus frequently dominate grassland areas (Anten and Hirose 1999; Grist 1999). In forest areas, species need to survive the presence of a dense tree canopy that changes light quality and quantity (Holmgren *et al.* 1997) and water availability. Additionally, they need to germinate and establish from beneath a thick litter layer (Sydes and Grime 1981a, 1981b). These high levels of stress during germination, establishment and growth, constrain the number of species in the understory vegetation. We expected higher

abundance of tolerant species in the forest areas. However, in our study there was one stress-tolerant species (i.e. S Grime strategy) with higher abundance in the forest, *G. odoratum*, which had also shown a positive response to the presence of litter (Donath and Eckstein 2008). Similar to the findings of Dierschke (1974), species such as shrub, annual forbs or geophytes were more common in the ecotone (i.e. P2 and P3 in the transects). Many shrubs and annual species are not able to survive below a dense forest canopy (Barbier *et al.* 2008; Denslow *et al.* 1990), which restricts their abundance to areas with only partial shade (Hastwell and Facelli 2003).

It is interesting to note that even short-time litter deposition on an established grassland had immediate negative effects on species richness and biomass. Although we expected that both variables would be affected, species richness was only reduced significantly when litter amount increased above $\sim 200\text{ g m}^{-2}$ in both the forest–grassland ecotone transects and the field experiment (Figures 1 and 3). In addition, the cover of the most common grassland species decreased significantly in plots covered with high litter amounts, but not at low litter amounts (except *A. elatius*, see Figure 4). The presence of small gaps at low litter amounts may help to explain the differences in vegetation cover, biomass or species richness between plots with low and high litter amounts. Some species may be able to grow through a low litter layer (Sydes and Grime 1981a), maintaining species richness, but their development (i.e. biomass) may be compromised (but see Dzwonko and Gawronski 2002).

It is generally assumed that natural ecotones have higher species diversity since they include species from adjacent ecosystem types (Hufkens *et al.* 2009). However, in anthropogenic

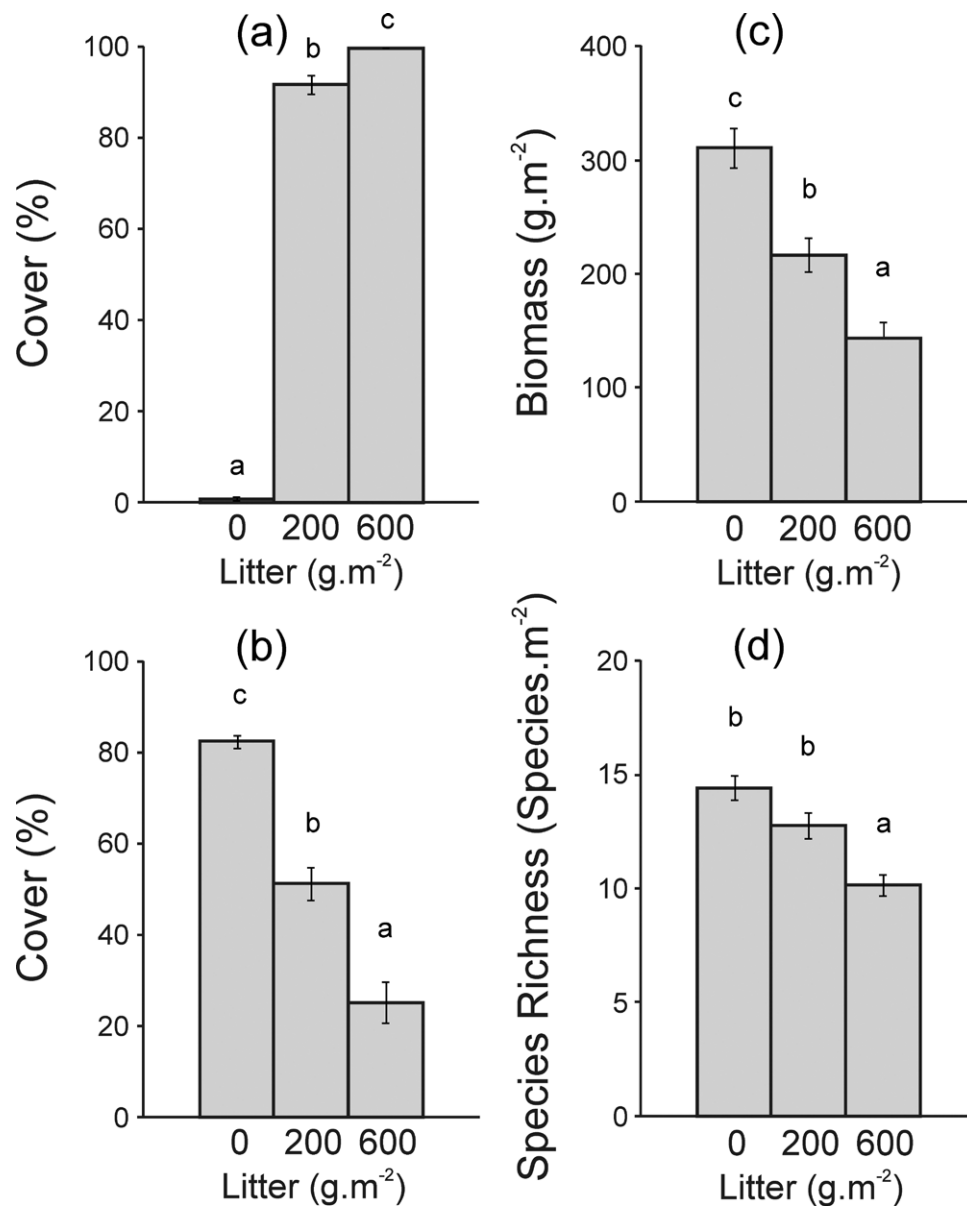


Figure 3: responses of litter cover (a), vegetation cover (b), vegetation biomass (c) and species richness (d) in plot with different litter amounts. Bars represent ± 1 SE. Different letters on bars indicate significantly different mean values (Tukey test at $P < 0.05$).

forest edges, as those studied here, boundaries between both ecosystems are sharply delimited (Dierschke 1974; Simmering et al. 2001) and thus the flora of each of these contrasting ecosystems is well delimited. As well the relation between litter amounts and vegetation composition in the field showed that litter has profound effects on species composition. In addition, the response of key grassland species in the field experiment just within a single growing season highlighted the rapid changes in vegetation composition that litter accumulation is able to induce. Nonetheless, in the long run, litter effects might be even stronger, since perennial plants with access to stored resources may display a lagged response of growth and fitness after litter accumulation took place.

Changes in microsite conditions

Our data showed that litter presence significantly reduced temperature amplitudes under high litter amounts. Many published works show that litter may affect temperature, reducing fluctuations and amplitude and extreme temperatures (Deutsch et al. 2010; Holmgren et al. 1997), even with a thin litter layer but without a live vegetation cover (Eckstein and Donath 2005; Jensen and Gutekunst 2003). Our data showed that these effects may vary when established vegetation is present, as evidenced by the same temperature amplitude between control and low litter treatment. This suggests that the impact of a litter layer changes depending on the presence of a vegetation canopy, which in turn changes conditions

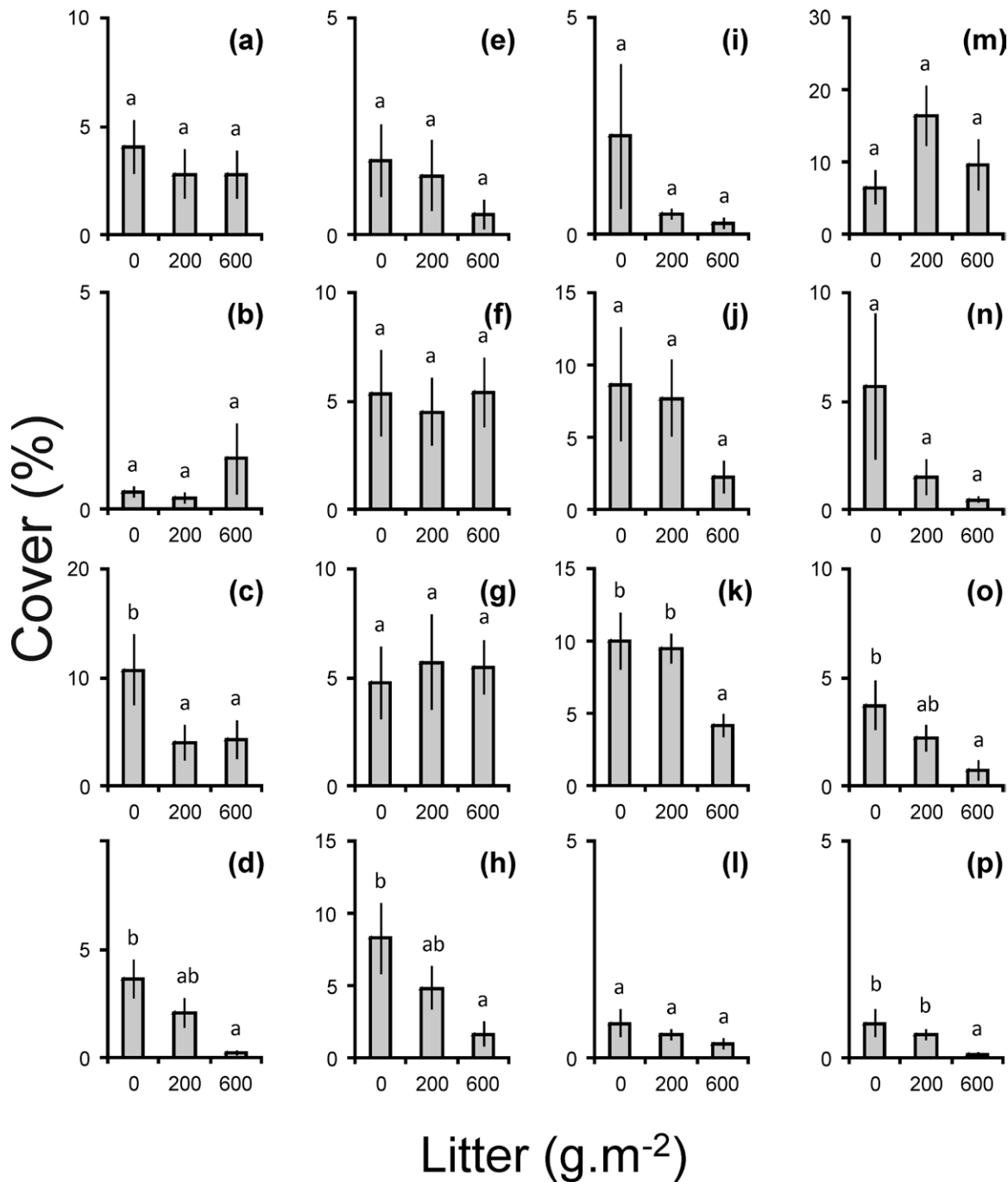


Figure 4: species cover of the most frequent species (frequency $\geq 50\%$) under different litter amounts. (a) *Achillea millefolium*, (b) *Ajuga reptans*, (c) *Arrhenatherum elatius*, (d) *Bromus hordeaceus*, (e) *Campanula rapunculus*, (f) *Crepis capillaris*, (g) *Dactylis glomerata*, (h) *Leontodon autumnalis*, (i) *Pimpinella saxifraga*, (j) *Plantago lanceolata*, (k) *Poa pratensis*, (l) *Ranunculus bulbosus*, (m) *Taraxacum officinalis*, (n) *Trifolium dubium*, (o) *Trisetum flavescens* and (p) *Veronica arvensis*. Bars represent ± 1 SE. Different letters on bars indicate significantly different mean values (Tukey test at $P < 0.05$).

near the soil surface (Schmiede *et al.* 2013). Although not statistically tested (due to the lack of variance), the difference in the number of frost days is remarkable. Since seedlings are frost sensitive (Fenner and Thompson 2005), differences in the frequency of frost days related to the litter cover of plots may affect the probability of species establishment and survival (Baltzinger *et al.* 2012).

The initial decomposition of litter, probably triggered by higher temperatures during spring and higher precipitation rates (see Figure S3), increase the availability of nutrients (Gartner and Cardon 2004; Sayer 2006). These initial conditions may explain the high availability of some nutrients during the first incubation period (e.g. Al, Ca, Mg, NO₃⁻N and P). During the second period, the values declined probably because many

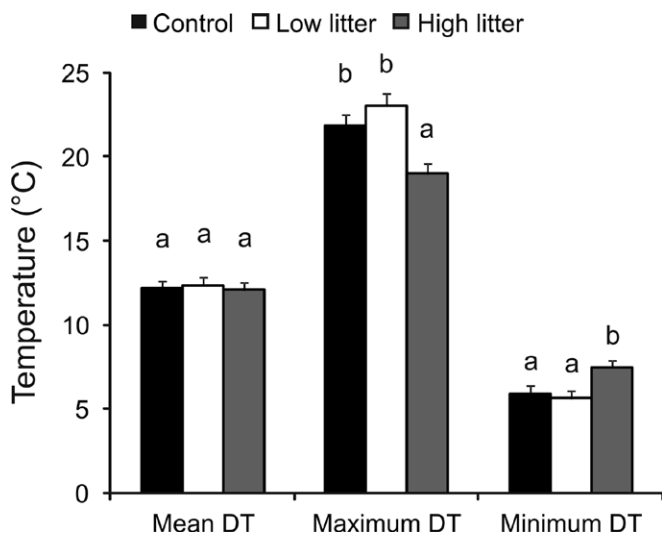


Figure 5: average mean, maximum and minimum daily temperature (DT) during the studied period. Bars are SE. Different letters on bars indicate significantly different mean values (Tukey test at $P < 0.05$).

of the easily decomposable materials had disappeared (Berg 2000). The rate of decomposition is related to temperature, precipitation and soil moisture (Aerts 1997; Hobbie 1996; Meentemeyer 1978), but also to the initial N concentration, C:N ratio and lignin:N ratio in the litter (Chadwick et al. 1998; Gartner and Cardon 2004). Generally, the most rapidly decomposable components in the litter are those with high N contents (Berg 2000; Gomez et al. 2002), which is reflected in our work by the increase in soil nitrate (NO_3^- -N) in plots with high litter amount during the first studied period. This decomposition rate is not only influenced by litter quality but also by litter quantity (Weatherly et al. 2003). A thicker litter layer may change micro environmental conditions, promoting a higher initial decomposition rate by microorganisms (Hobbie 1996; Zhang and Zak 1995). A thinner, or absent, litter layer may lead to heat stress or desiccation of decomposers, reducing decomposition rates (Henry et al. 2008), while the absence of litter increases the chances of leaching from the soil, particularly for nitrogen (Sayer 2006). Higher soil moisture, due to lower temperatures, during the first incubation period (mean \pm

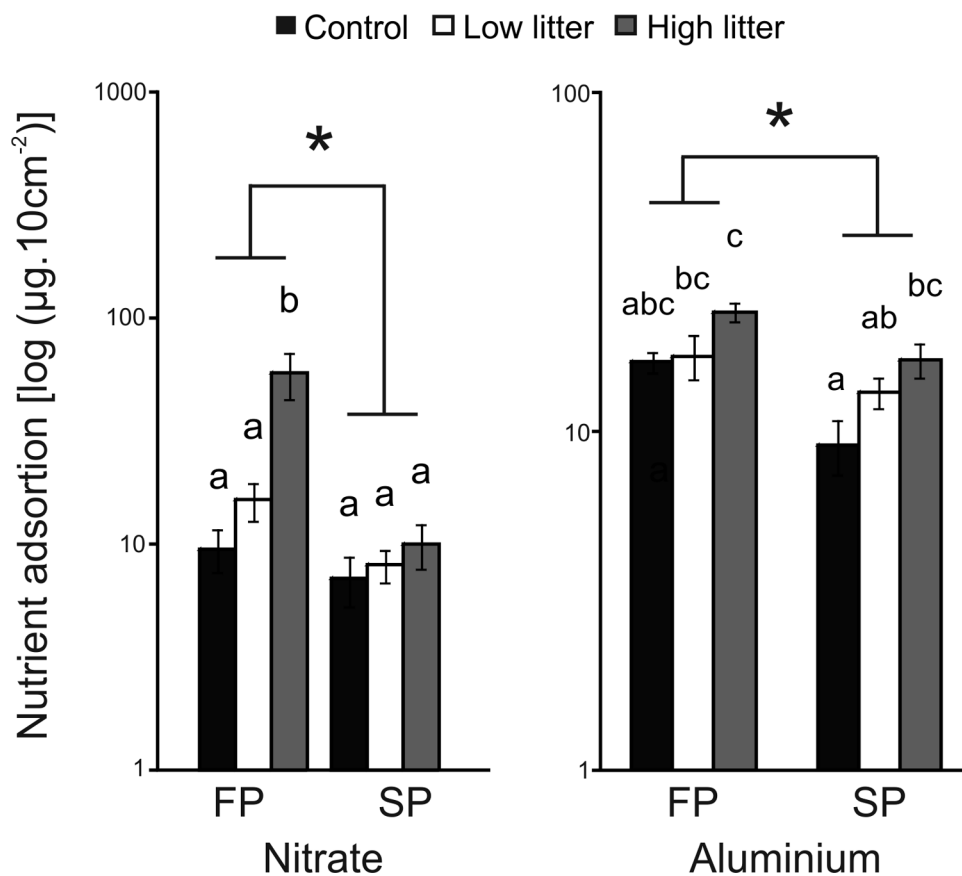


Figure 6: adsorption of nitrate (NO_3^- -N, left panel) and aluminium (right panel) during two consecutive 4-week incubation periods in grassland soil covered by different amounts of tree litter (control: 0, low: 200 and high: 600 g m^{-2}). Bars represent ± 1 SE. Different letters on bars are significantly different mean values (Tukey test at $P < 0.05$). Statistical differences between first (FP) and second (SP) incubation period is indicated as $*P < 0.05$. Note the logarithmic scale of the y-axis.

SE: 13.6 ± 0.16 vs. $15.6 \pm 0.09^\circ\text{C}$; first and second incubation period, respectively, see [Figure S4](#), see online supplementary material) and lower competition levels (see [Figure 3](#)) may also be responsible for higher nitrogen adsorption in the probes. In contrast, lower adsorption of Ca, Mg and P in the second incubation period may be due to lower soil moisture conditions (resulting from higher temperature), which strongly affect the activity of these ions in soil solution (Eric Bremer, Western Ag Innovation, personal communication). The increase in Al under tree litter is a signal of acid hydrolysis on silicate ([Amiotti et al. 2000](#); [Langenbruch et al. 2012](#); [Paluch and Gruba 2012](#); [Sayer 2006](#)), being an indication of a microsite reduction in pH ([Langenbruch et al. 2012](#)). Unfortunately, we did not measure soil pH in this experiment. However, [Eckstein et al. \(2012\)](#) reported lower pH values for forest edges than grassland areas in the same experimental field ($\text{pH}_{\text{water}} = 3.57$ and 4.89 for forest edge and grassland, respectively). Changes in microsite conditions should be monitored during a longer period, especially during winter, and with more precision and details in order to obtain some new insights that may help to predict changes in grasslands ecosystems invaded by tree species, a common process worldwide ([Bren 1992](#); [Brown and Archer 1999](#); [Chaneton et al. 2012](#); [Mazia et al. 2001](#); [Zalba et al. 2008](#)).

CONCLUSIONS

Since forest–grassland ecotones in the study area are anthropogenic, they show sharp boundaries and are dominated by different vegetation types with some of them restricted to a very small area within the ecotone. In the field experiment, litter accumulation affected several key species of mesic grasslands, leading to significantly decreased growth. This might, in turn, result in changes in the species composition once litter accumulation reaches high values ($>200 \text{ g m}^{-2}$), and also changes in soil conditions, beginning an acidification process. Along forest edges, this could be a rapid and common process that leads to the replacement of typical grassland species by woody or stress-tolerant species.

SUPPLEMENTARY MATERIAL

[Supplementary material](#) is available at *Journal of Plant Ecology* online.

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