

# Does vegetation complexity affect host plant chemistry, and thus multitrophic interactions, in a human-altered landscape?

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**Abstract** Anthropogenic land use may shape vegetation composition and affect trophic interactions by altering concentrations of host plant metabolites. Here, we investigated the hypotheses that: (1) plant N and defensive secondary metabolite contents of the herb *Plantago lanceolata* are affected by land use intensity (LUI) and the surrounding vegetation composition (=plant species richness and *P. lanceolata* density), and that (2) changes in plant chemistry affect abundances of the herbivorous weevils *Mecinus pascuorum* and *Mecinus labilis*, as well as their larval parasitoid *Mesopolobus incultus*, in the field. We determined plant species richness, *P. lanceolata* density, and abundances of the herbivores and the parasitoid in 77 grassland plots differing in LUI index in three regions across Germany. We also measured the N and secondary

metabolite [the iridoid glycosides (IGs) aucubin and catalpol] contents of *P. lanceolata* leaves. Mixed-model analysis revealed that: (1) concentrations of leaf IGs were positively correlated with plant species richness; leaf N content was positively correlated with the LUI index. Furthermore: (2) herbivore abundance was not related to IG concentrations, but correlated negatively with leaf N content. Parasitoid abundance correlated positively only with host abundance over the three regions. Structural equation models revealed a positive impact of IG concentrations on parasitoid abundance in one region. We conclude that changes in plant chemistry due to land use and/or vegetation composition may affect higher trophic levels and that the manifestation of these effects may depend on local biotic or abiotic features of the landscape.

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## Introduction

Multitrophic interactions are important drivers of ecosystem stability and functioning, especially because they can influence nutrient and energy cycling (reviewed by Weisser and Siemann 2004; Schmitz 2010). Ecosystem stability is endangered by loss of biodiversity (Balvanera et al. 2006) caused by the ongoing process of land use intensification in numerous habitats (Foley et al. 2005). A decline in the abundance of plant species may also affect the abundances of members of higher trophic levels (Joshi et al. 2004; Petermann et al. 2010).

Vegetation composition in terms of plant species diversity and conspecific plant density determines the abiotic

and biotic environment of an individual plant and, thus, its growth, development, and primary and secondary metabolite concentrations (Cipollini and Bergelson 2001; Barbosa et al. 2009; Broz et al. 2010; Scherling et al. 2010). For example, plant neighbours of an individual plant can change the availability of nutrients in the habitat or alter the microclimate that influences light intensity and the temperature within a microhabitat, and thus may also change competition levels for this focal plant (Barbosa et al. 2009). Plants may also sense their neighbours via airborne plant-emitted signals, or soluble or volatile compounds in the soil, or the mycorrhizal system, and respond with changes in primary or secondary metabolite concentrations (Heil and Karban 2010; Kegge and Pierik 2010; Selosse et al. 2006). Furthermore, land use practices (mowing, grazing by cattle, and fertiliser application) influence the vegetation composition (Spiegelberger et al. 2006) as well as primary and secondary metabolite concentrations in plants (Prudic et al. 2005).

Changes in plant metabolite allocation and concentrations can shape multitrophic interactions by altering the performance and behaviour of organisms of the second (Schoonhoven et al. 2005) and the third (Gols and Harvey 2009) trophic levels. In general, plant N content as a factor indicating nutrient quality is proposed to correlate positively with herbivore performance (and thus with herbivore abundance) (Mattson 1980; White 1993; Throop and Ler dau 2004). However, plants with high N content can also negatively affect the fitness of herbivores (Joern and Behmer 1998; Fischer and Fiedler 2000). In addition to a direct influence of the plant's nutrient content on herbivore performance, plant nutrients may also affect herbivores by their indirect effect on the third trophic level; the indirect effect may act via a trophic cascade mediated by host or prey quality, thereby leading to altered parasitism rates (Thompson 1999, Sarfraz et al. 2009a).

Secondary metabolites of plants may function as defensive compounds against herbivores. Numerous herbivorous insects have in turn evolved ways to handle secondary plant metabolites by sequestering or detoxifying them (reviewed by Opitz and Müller 2009). Like the plant's primary metabolite content, the secondary metabolite concentrations of a host plant may also affect the third trophic level by influencing host or prey quality, and thus the nutritional value of a resource exploited by parasitoids and predators (Williams 1999; Kos et al. 2011). Furthermore, parasitoids and predators are known to use secondary plant metabolites as cues that inform about the presence and quality of hosts or prey. Therefore, they might be affected by the secondary metabolite content of a host plant whilst host foraging (Dicke and van Poecke 2002; Poelman et al. 2008; Gols et al. 2009).

Until now, the effects of plant species diversity on concentrations of secondary plant metabolites that are

characteristic anti-herbivore defensive compounds have been investigated only by Mraja et al. (2011); they showed that plant species richness affected concentrations of secondary plant metabolites of the perennial herb *Plantago lanceolata* L. (Plantaginaceae) that is native to Europe and widespread in a variety of habitats (Schubert and Vent 1990). Characteristic secondary metabolites of *P. lanceolata* are iridoid glycosides (IGs), especially aucubin and the more toxic catalpol (Bowers 1991). The IGs are monoterpene derivatives and toxic for many herbivores (Bowers 1991; Dobler et al. 2011). Mraja et al. (2011) showed that concentrations of aucubin decreased and those of catalpol increased when plant species richness increased. Their study was conducted in experimental grassland plots where plant species were sown and weeded to keep plant species richness constant. In order to investigate whether the patterns observed can also be detected in a natural setting, field studies in different regions that vary in their landscape composition and abiotic parameters are needed (Fischer et al. 2010).

We conducted a large-scale field study in various geographic regions within a human-altered landscape in Germany which addressed the question of how land use, plant species richness and density of *P. lanceolata* affect IG concentrations and leaf N content of *P. lanceolata*. N is usually a crucial factor in insect nutrition (Schoonhoven et al. 2005) and may determine, in addition to plant secondary metabolite concentrations, herbivore abundances. Changes in plant chemistry that are due to land use and/or vegetation composition (as defined by plant species richness and conspecific plant density) may also affect higher trophic levels. Therefore, we studied how the abundances of two herbivorous insects and their parasitoid in the field were affected by host plant chemistry and vegetation composition. The herbivorous insects studied were *Mecinus pascuorum* (Gyllenhal) and *Mecinus labilis* (Herbst) (both Coleoptera: Curculionidae), which are specialists on IG-containing plants (Freude et al. 1983). They feed on *P. lanceolata* stalks and leaves during their adult phase; females oviposit into seeds, and larvae develop inside the seeds. The parasitic insect species studied was the larval parasitoid *Mesopolobus incultus* (Walker) (Hymenoptera: Pteromalidae), which attacks *Mecinus* larvae inside the seeds (Mohd Norowi et al. 2000).

In summary, we tested the hypotheses that:

1. Land use intensity (LUI) and vegetation composition (plant species richness and *P. lanceolata* density) affect N and IG (aucubin and catalpol) leaf contents of *P. lanceolata*.
2. Plant chemistry changes impact the abundances of the herbivores *Mecinus pascuorum* and *Mecinus labilis*, and of the parasitoid *Mesopolobus incultus*.

## Materials and methods

### Study sites

This study was part of the German priority project Biodiversity Exploratories which is described in detail by Fischer et al. (2010). Samples were taken in three geographic regions (exploratories) in Germany (from north to south): the Schorfheide-Chorin Biosphere Reserve (centre, ~52°54'N, 3°52'E), a young glacial landscape in north-eastern Germany; the Hainich-Dün National Park (centre, ~51°6'N, 10°23'E), situated in the hilly country in central Germany; and the Schwäbische Alb Biosphere Area (centre, ~48°24'N, 9°12'E), a sub-mountainous area in south-western Germany. The three regions across Germany differ in environmental conditions such as precipitation (from north to south, 500–600, 500–800, 700–1000 mm), altitude (3–140, 285–550, 460–860 m) and annual mean temperature (8–8.5, 6.5–8, 6–7 °C) (Fischer et al. 2010). In each region, 50 grassland plots (50 m × 50 m) were established for biodiversity research. A plot is almost homogeneous with respect to soil type and vegetation properties. Plot edge effects were avoided by choosing plot areas where vegetation along the plot borders was similar to the vegetation within the plot, and the nearest forest edge had a distance of 30 m to the plot border (Fischer et al. 2010). The grassland sites capture a land use gradient and concomitant differences in plant species richness.

Land use practice is typified as meadows, pastures and mown pastures with or without fertiliser application. According to Blüthgen et al. (2012) LUI can be expressed as a continuous variable, the LUI index. This index is calculated by different measures for land use: amount of fertiliser (kg N ha<sup>-1</sup>), frequency of mowing (year<sup>-1</sup>), and livestock density (livestock units × days ha<sup>-1</sup>). Due to the occurrence of the host plant, *P. lanceolata*, we could collect data in 21 plots in Schorfheide-Chorin, 22 in Hainich-Dün, and 34 in the Schwäbische Alb. The plots of each region showed an almost representative land use continuum with most plots in the range of 0.5–2.5 (LUI index). In Schorfheide-Chorin and the Schwäbische Alb, there were also a few plots outside this range (Table S1, supplement).

### Sampling of leaf material

Within each plot we sampled ten randomly chosen focal *P. lanceolata* plants distributed across the plot using a grid across the plot and random numbers. In the randomly chosen grid fields we sampled leaf material from the plant that was closest to a grid border. Plant species richness and *P. lanceolata* density defined as the vertical covering of *P. lanceolata* were measured in circles (15-cm radii) around each focal plant once in June 2008. The distance between

focal plants was always chosen in a way that circles around focal plants did not overlap.

In order to sample plant material for later chemical analyses, we did not cut any stalks or inflorescences of *P. lanceolata* since these plant parts are inhabited by the *Mecinus* weevils and their parasitoids. Cutting these plant parts could have influenced abundances of weevils and parasitoids within a plot. Instead we sampled leaf material to measure N and IG contents of *P. lanceolata*. As IG concentrations of different plant organs are positively correlated (Darrow and Bowers 1999), we used leaf IG content as a proxy for IG content in stalks and inflorescences. Leaf N content can be used as a proxy for the nutritional quality of a plant (Quintero and Bowers 2012) and served here as an indicator for the nutrient availability in the habitat. The metabolite composition of leaves varies according to factors like leaf age, genotype (Bowers and Stamp 1992), ecotype (Marak et al. 2000), and herbivory (Bowers and Stamp 1993; Darrow and Bowers 1999). Nevertheless, we sampled mid-aged leaves from plants regardless of leaf damage and geno- or ecotype; leaf samples were taken from ten plants per plot that were chosen according to a randomly applied route pattern; thus, we obtained a set of leaves that was representative for the entire plot. Leaves were harvested and immediately stored on dry ice. In the laboratory leaves were kept at -30 °C until sample preparation.

### Determination of insect abundances

To determine the abundances of the herbivores *Mecinus pascuorum* and *Mecinus labilis* and the larval parasitoid *Mesopolobus incultus*, we collected 100 randomly chosen (see above) *P. lanceolata* inflorescences per plot in July and August 2008. Inflorescences of *P. lanceolata* were kept in plastic boxes (17.0 × 12.5 × 5.6 cm) with a fine-meshed gauze (0.12 mm) top cover under constant conditions (11-h:13-h light:dark, temperature: 22 °C, 50 % relative humidity). Adult weevils and parasitoids that emerged from the inflorescences in August and September 2008 were identified and counted. The emergence data were used as estimators for field abundance.

### Chemical analysis

Prior to analyses, frozen leaf samples that were collected in the field and stored as described above were ground in liquid N<sub>2</sub> in the laboratory. After grinding, the fine powder was lyophilised.

N content of the dried and ground plant material was analysed by an elemental analyser (Euro EA 3000 elemental analyser; EuroVector, Milan) and is given in % dry weight (DW).

For IG analysis, we added 50  $\mu\text{l}$  of 20 mM  $\text{KH}_2\text{PO}_4$  and 1 ml methanol to 20 mg lyophilised leaf material. The mixture was shaken at 40 °C and 1500 r.p.m. for 4 h in a rotary shaker (Thermomixer Comfort; Eppendorf, Hamburg) and centrifuged for 3–4 min at 10,000 r.p.m. The supernatant (100  $\mu\text{l}$ ) was concentrated to dryness under a  $\text{N}_2$  stream. Finally, we reconstituted the concentrate in 100  $\mu\text{l}$  of 20 mM  $\text{KH}_2\text{PO}_4$  and analysed the solution by high performance liquid chromatography (HPLC) with diode array detection.

The Shimadzu HPLC system (Shimadzu, Duisburg, Germany) consisted of two LC-20AD pumps, a CBM-20A system controller, a manual injection system, and a Spherisorb ODS-2 analytical column (3  $\mu\text{m}$ ; 60 mm  $\times$  4.6 mm) with a guard column (same material, 10 mm  $\times$  4.6 mm). The solvents were 20 mM  $\text{KH}_2\text{PO}_4$  (A) and acetonitrile (B). We used the following gradient: 0–5 min, 5 % of B; 5–9 min, 5–50 % of B. The flow rate was 0.5 ml  $\text{min}^{-1}$ . The injection volume was 20  $\mu\text{l}$ . The IGs were detected with a photodiode array detector SPD-M20A (190–300 nm). Retention times and ultra-violet spectra were compared with reference compounds (aucubin plus catalpol, purity  $\geq 99$  %; Phytoplan Diehm and Neuberger, Heidelberg) by the software Chromatography data system LC-Solution (Shimadzu). For quantification, peak areas of aucubin and catalpol were determined at 210 nm. Amounts of aucubin and catalpol were calculated by calibration with the respective reference compounds. Quantities of single IGs, as well as the sum of both compounds (total IGs), are given in mg/g leaf DW.

### Statistical analysis

Field data were analysed using linear mixed models and generalised linear mixed models. The models are based on mean values per plot for *P. lanceolata* density and mean number of plant species (=plant species richness) per plot. Furthermore, the numbers of weevils (or parasitoids) that emerged per plot were used for the models. Additionally, the N and IG contents that were determined for leaf material samples collected per plot were incorporated into the model as plot values. All calculations were performed using R (version 2.12.1; R Development Core Team 2010). Models were calculated with the lmer function (package lme4 version 0.999375-37). The region was used as a random effect. Fixed effects with non-normal distribution were ln transformed to stabilize variance (Crawley 2007). A term (+1) was added before transformation if necessary. For plant N and IG contents as response variables we used a Gaussian error distribution (link = identity) and fitted the models by maximum likelihood. Model assumptions were checked graphically by forming normal  $q$ - $q$  plots. For the insect abundance data, as response we used a Poisson error

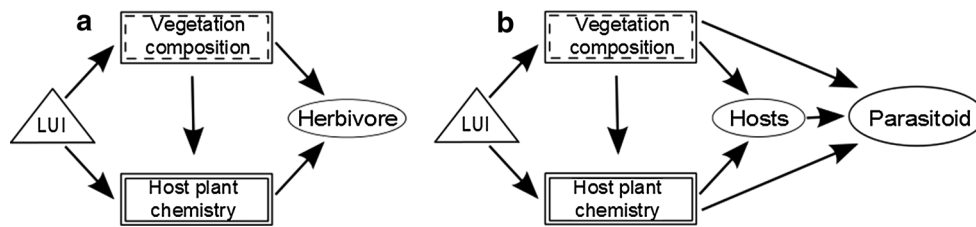
distribution (link = log) and the Laplace approximation (Crawley 2007). Furthermore, as the data showed overdispersion, we added an individual-based random effect (Elston et al. 2001). We started with the full model and discarded terms that were not significantly different from zero. Model comparison was carried out using the Akaike information criterion (AIC) (Burnham and Anderson 2002) until the point at which a minimal adequate model was found (with the AIC no longer decreasing) or all variables proving significantly different from zero.

To analyse the effect of the LUI index on vegetation composition we included plant species richness and *P. lanceolata* density, respectively, as response variables and added the LUI index as a fixed effect. To elucidate the effect of the LUI index and vegetation composition on plant chemistry (N, total IG, aucubin, and catalpol) we used the following fixed effects: plant species richness, *P. lanceolata* density, LUI index, region, and N content. To determine the effect of plant chemistry changes by surrounding vegetation on the multitrophic interactions we included the following fixed effects in the model: N, total IGs, ratio of catalpol to total IGs, *P. lanceolata* density, plant species richness, and host abundance. Since host availability, host plant density and plant species richness determine herbivore and parasitoid abundance (e.g. Root 1973; Sheehan 1986) we included these variables in the analysis as covariates.

Furthermore, we set up structural equation models (SEMs) for each region using partial least squares (PLS) regression with the program Warp PLS 3.0 (Kock 2012). The PLS regression was chosen over covariance-based approaches because it suited our small sample size and, compared to covariance structure analysis, can accommodate both reflective and formative scales more easily. Moreover, PLS does not require any a priori distributional assumptions (Chin and Newsted 1999). We present individual standardised path coefficients ( $\beta$ ), partial model fit scores ( $R^2$ ), and overall model  $P$ -values calculated by resampling estimations coupled with Bonferroni-like corrections (Kock 2010). To validate the models three model-fit indices [average path coefficient (APC), average R-squared (ARS), and average variance inflation factor (AVIF)] were calculated for each region. For model fit, it is recommended that  $P$ -values for APC and ARS are both lower than 0.05 (i.e. significance at the 0.05 level). The AVIF index controls for multicollinearity and should be below 5 (Kock 2010).

In the SEM analysis we set paths from vegetation composition (plant species richness and *P. lanceolata* density) and plant chemistry parameters (total IGs and N) directly to herbivore abundance (or parasitoid abundance); furthermore, we included the possible effects of the LUI index on vegetation composition (plant species richness and *P. lanceolata* density) and plant chemistry parameters (total IGs and N), as well as the effects of vegetation composition





**Fig. 1** Initial full structural equation models (SEMs) of potential relationships explaining **a** herbivore and **b** parasitoid abundance. Vegetation composition includes *Plantago lanceolata* density and plant species richness. Host plant chemistry comprises total iridoid glycoside (IG) and N contents in *P. lanceolata* leaves. Model A was

calculated for both herbivore species (*Mecinus pascuorum* and *Mecinus labilis*) and for each region separately. Model B was calculated to explain parasitoid (*Mesopolobus incultus*) abundance; here both herbivore species were included as one host parameter. LUI Land use intensity

on plant chemistry (Fig. 1a). For analysis of the parasitoid abundance data, a path via host abundance was added (Fig. 1b).

## Results

### Effect of LUI and vegetation composition on *P. lanceolata* metabolites

Over all plots and regions the LUI index and plant species richness correlated negatively ( $t = -4.087$ ,  $df = 73$ ,  $P < 0.001$ ), whereas no correlation was found between the LUI index and *P. lanceolata* density ( $t = 0.910$ ,  $df = 73$ ,  $P = \text{non-significant}$ ). Thus, higher LUI led to a reduction in plant species richness.

The leaf N content of *P. lanceolata* ranged on average from 1.9 % to 2.3 % DW (Table S2, supplement) and was slightly higher in Schorfheide-Chorin than in Hainich-Dün (Table 1). Leaf N content did not correlate with plant species richness or *P. lanceolata* density, but correlated positively with the LUI index (Fig. 2a), indicating that more intense land use leads to a higher leaf N content.

The total IG concentration in *P. lanceolata* leaves ranged in average from 36.3 to 41.5 mg/g (Table S2, supplement). The concentrations of aucubin and catalpol, as well as of total IGs, correlated positively with plant species richness (Fig. 2b; Table 1). The LUI index and *P. lanceolata* density had no effect on the IG concentrations of *P. lanceolata* leaves. Only the catalpol concentrations differed between the regions and were slightly higher in Hainich-Dün than in the Schwäbische Alb (Table 1).

### Plant metabolites and higher trophic levels

The abundance of both herbivore species was highest in Schorfheide-Chorin. In this region, abundances of the weevil *M. pascuorum* were higher than those of *M. labilis*. However, in the Schwäbische Alb, abundances of the

weevil *M. labilis* were higher than those of *M. pascuorum* (Table S3, supplement). Over all three regions the abundances of both herbivores correlated negatively with host plant N content. Abundances of neither herbivore species were correlated with the concentrations of total IGs nor with the ratio of catalpol to total IGs (Table 2). Furthermore, the abundance of the weevil *M. pascuorum* correlated positively with plant species richness. When analysing data obtained in the three regions separately, significant SEMs for both herbivore species were only obtained for Hainich-Dün [*M. pascuorum* APC = 0.536 ( $P < 0.001$ ), ARS = 0.655 ( $P = 0.008$ ), AVIF = 1.019; *M. labilis* APC = 0.883 ( $P = 0.002$ ), ARS = 0.781 ( $P = 0.014$ ), AVIF = 1.000]. In this region, leaf N content had a negative influence on abundances of both herbivore species (*M. pascuorum*,  $R^2 = 0.65$ ,  $\beta = -0.55$ ,  $P < 0.01$ ; *M. labilis*,  $R^2 = 0.78$ ,  $\beta = -0.88$ ,  $P < 0.01$ ). Furthermore, *P. lanceolata* density correlated negatively with *M. pascuorum* abundance in this region ( $\beta = -0.52$ ,  $P < 0.05$ ).

The abundance of the parasitoid *M. incultus* followed the abundance pattern of its hosts, with greatest abundance in Schorfheide-Chorin and least in the Schwäbische Alb. Over all three regions leaf N and IG concentrations did not explain parasitoid abundance, but herbivore abundance correlated positively with parasitoid abundance (Table 2). When analysing parasitoid abundance data for the three regions separately by SEMs, we identified several causal relationships between plant species richness or the LUI index, host plant chemistry and higher trophic levels (Fig. 3). The initial (full) models were not significant [Schorfheide-Chorin, APC = 0.296 ( $P < 0.001$ ), ARS = 0.328 ( $P = 3.753$ ), AVIF = 1.289; Hainich-Dün, APC = 0.267 ( $P = 0.007$ ), ARS = 0.402 ( $P = 0.324$ ), AVIF = 1.576; Schwäbische Alb, APC = 0.266 ( $P < 0.001$ ), ARS = 0.237 ( $P = 7.125$ ), AVIF = 1.276; see supplemental material Fig. S1]. By reducing variables and links between variables we obtained significant SEMs for each of the three regions (Fig. 3). A substantial portion of variability in parasitoid abundance was

**Table 1** Effect of vegetation composition (plant species richness plus *Plantago lanceolata* density), the land use intensity (LUI) index (and N content) and the region [Schorfheide-Chorin (SCH), Hainich-Dün (HAD), Schwäbische Alb (ALB)] on total iridoid glycosides (IGs) (aucubin plus catalpol), on aucubin and catalpol separately, and on N content of *P. lanceolata* leaves

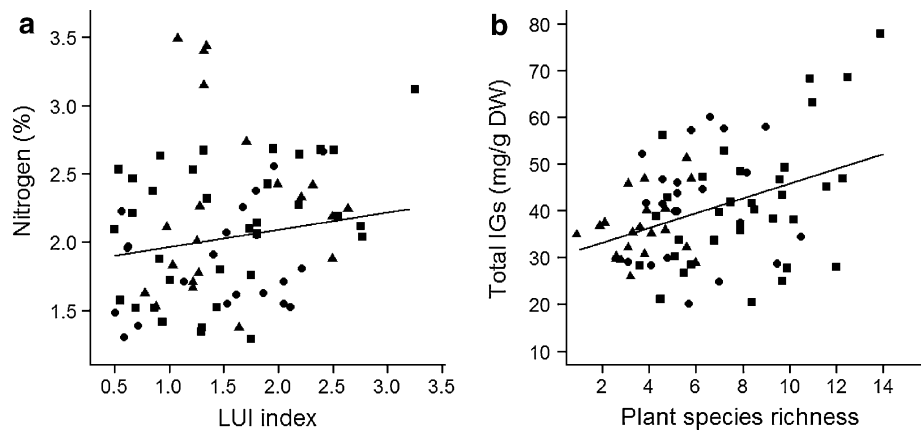
Explanatory variables	Total IG (mg/g dry weight)			Aucubin (mg/g dry weight)			Catalpol (mg/g dry weight)			N (%)						
	$\beta$	SE	<i>t</i> -value	<i>P</i>	$\beta$	SE	<i>t</i> -value	<i>P</i>	$\beta$	SE	<i>t</i> -value	<i>P</i>				
Intercept (HAI)	29.985	2.957	10.142	<0.001	14.964	1.979	7.561	<0.001	16.622	2.715	6.122	<0.001	1.6305	0.1578	10.336	<0.001
Plant species richness	1.574	0.424	3.716	<0.001 (+)	0.869	0.284	3.064	<0.01 (+)	1.044	0.373	2.799	<0.01 (+)	–	–	–	–
<i>P. lanceolata</i> density	– <sup>a</sup>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LUI index	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
N	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Region: SCH	–	–	–	–	–	–	–	–	–3.393	2.334	–1.453	>0.05	0.3583	0.1477	2.425	<0.01 (+)
Region: ALB	–	–	–	–	–	–	–	–	–6.406	2.099	–3.052	<0.01 (–)	0.2044	0.1325	1.543	>0.05
AIC full model	–	–	598.1	–	–	–	532.1	–	–	–	535.0	–	–	–	120.9	–
AIC minimal model	–	–	590.8	–	–	–	529.0	–	–	–	531.9	–	–	–	118.5	–
den $df$	–	–	75	–	–	–	75	–	–	–	73	–	–	–	73	–

Results of the linear mixed models are shown: estimates ( $\beta$ ), SE, *t*-values, and significant effects of the minimal adequate model [evaluated by Akaike information criterion (AIC)], and denominator *df* (*den $df$* ) are given. *P*-values are in italic if significant; directions of effects are shown in *parentheses*. Seventy-seven plots were included in the analysis

NA Not included in the full model

<sup>a</sup> Indicates excluded by model simplification

**Fig. 2** The effect of **a** the LUI index on N content and **b** plant species richness on total iridoid glycoside (IG) concentrations in *P. lanceolata*. Triangles represent data of Schorfheide-Chorin, circles data of Hainich-Dün, and squares data of the Schwäbische Alb. Lines represent significant relationships obtained from the linear mixed model



**Table 2** Effect of host plant chemistry (N and IG contents in *P. lanceolata* leaves, ratio of catalpol to total IGs) and vegetation composition (plant species richness and *P. lanceolata* density) on the abun-

dances of the herbivores *Mecinus pascuorum* and *Mecinus labilis* and their parasitoid *Mesopolobus incultus*

Explanatory variables	Abundance <i>Mecinus pascuorum</i>				Abundance <i>Mecinus labilis</i>				Abundance <i>Mesopolobus incultus</i>			
	$\beta$	SE	z value	P	$\beta$	SE	z value	P	$\beta$	SE	z value	P
Intercept	0.498	2.363	0.211	>0.05	2.428	1.791	1.356	>0.05	-0.495	0.273	-1.812	<0.1
N content	-1.870	0.688	-2.718	<0.01 (-)	-1.912	0.891	-2.145	<0.05 (-)	-	-	-	-
Total IGs	<sup>b</sup>	-	-	-	-	-	-	-	-	-	-	-
Catalpol/total IGs	-	-	-	-	-	-	-	-	-	-	-	-
Plant species richness	0.432	0.164	2.632	<0.01 (+)	-	-	-	-	-	-	-	-
<i>P. lanceolata</i> density	-	-	-	-	-	-	-	-	-	-	-	-
Host abundance <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	1.119	0.119	9.373	<0.001 (+)
AIC full model	217.3				196.0				252.0			
AIC minimal model	212.1				190.1				245.2			
dendf	74				75				75			

The effect of host abundance on parasitoid abundance was also included. Results of the generalised linear mixed model are shown: estimates ( $\beta$ ), SE, z-values, and significant effects of the minimal adequate model (evaluated by AIC), and dendf are given. P-values are in italic if significant; directions of effects are shown in parentheses. Seventy-seven plots were included in the analysis. For other abbreviations, see Table 1

<sup>a</sup> Ln transformed

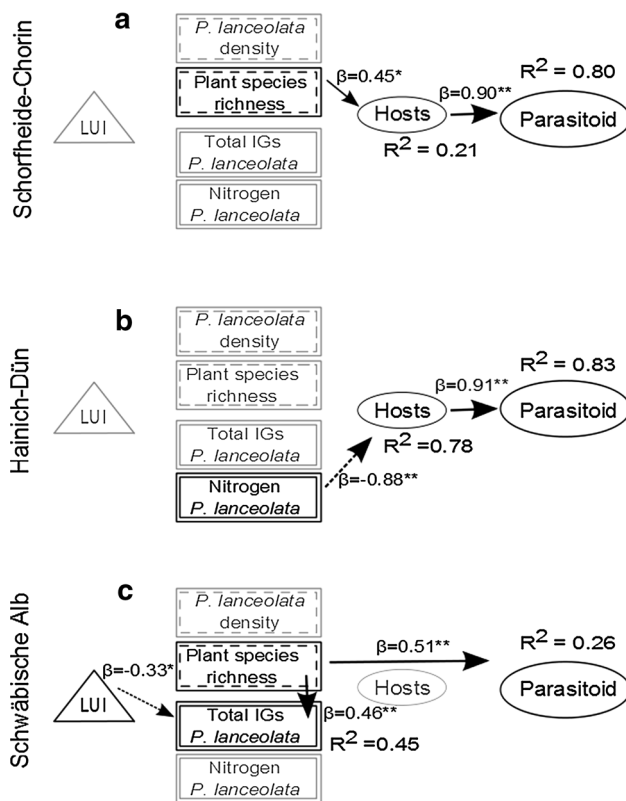
<sup>b</sup> Excluded by model simplification

explained by the obtained models in Schorfheide-Chorin ( $R^2 = 0.80$ ) and Hainich-Dün ( $R^2 = 0.83$ ), but not in the Schwäbische Alb ( $R^2 = 0.26$ ). The model for Schorfheide-Chorin [APC = 0.675 ( $P < 0.001$ ), ARS = 0.504 ( $P = 0.023$ ), AVIF = 1.000] revealed a positive effect of plant species richness on host abundance (=abundance of both herbivore species) that affected the abundance of the parasitoid positively. In Hainich-Dün [APC = 0.896 ( $P < 0.001$ ), ARS = 0.804 ( $P = 0.003$ ), AVIF = 1.000] leaf N content affected host abundance negatively, which in turn influenced the parasitoid abundance positively. In the Schwäbische Alb [APC = 0.434 ( $P < 0.001$ ), ARS = 0.357 ( $P = 0.024$ ), AVIF = 1.230], host abundance did not play a role in parasitoid abundance, and the model indicates a positive effect of total IG concentrations on parasitoid

abundance. In addition, the LUI index had a negative effect, and plant species richness a positive effect, on total IG concentrations (Fig. 3).

### Discussion

Our field study addressed the question whether changes in LUI and vegetation composition (plant species richness and *P. lanceolata* density) induce phytochemical changes in *P. lanceolata* and thus impact the abundance of herbivorous insects and parasitoids of this host plant. We demonstrate that *P. lanceolata* chemistry may change when LUI and composition of the surrounding vegetation changes. These changes in host plant chemistry affected abundances



**Fig. 3** Minimal significant SEMs including the host (*Mecinus pascuorum* plus *Mecinus labilis*) and the parasitoid (*Mesopolobus incultus*) abundances are shown for the three regions from north to south: Schorfheide-Chorin (a), Hainich-Dün (b), Schwäbische Alb (c). Vegetation composition parameters (*P. lanceolata* density and plant species richness) are shown in dashed framed boxes, host plant chemistry parameters (total IGs and N contents in *P. lanceolata* leaves) are represented in solid framed boxes. Dashed arrows indicate negative, and solid arrows positive, significant relationships. Partial model-fit scores ( $R^2$ ), path coefficients ( $\beta$ ) and the strength of significance are given (\* $P < 0.05$ , \*\* $P < 0.01$ ). Variables in grey are excluded from the minimal significant model. For abbreviations, see Figs. 1 and 2

of members from higher trophic levels in different ways. Abundances of the herbivorous weevils were more strongly affected by plant N content, whereas parasitoid abundance was influenced by the availability of the host (weevil abundance) and the secondary metabolite content (total IG concentration) of the host plant.

### Effect of vegetation composition on *P. lanceolata* secondary metabolites

Concentrations of the IGs aucubin and catalpol in *P. lanceolata* leaves correlated positively with plant species richness. Plant–plant interactions and interspecific competition may explain this positive correlation. Since plant IGs can act allelopathically against heterospecific plant neighbours and inhibit seed germination (Adam et al. 1979; Page et al.

1994; Pardo et al. 1998), the presence of heterospecific competitors might lead to an increase of IG concentrations in order to lower competition. Since *P. lanceolata* roots and root exudates contain IGs (Wurst et al. 2010) [and their concentrations correlate positively with IG concentrations in leaves (De Deyn et al. 2009)], root-released IGs might control interspecific competition. The *Plantago* individuals might be informed about the presence of heterospecifics either via airborne plant-emitted volatiles, compounds in the soil, or via the mycorrhizal system (Heil and Karban 2010; Kegge and Pierik 2010; Selosse et al. 2006) and respond with the production of IGs. Future studies need to elucidate how *P. lanceolata* perceives the presence of heterospecific plants and whether these compounds have a function in plant–plant interactions.

Our findings contrast with recent field and/or greenhouse studies by Broz et al. (2010) and Mraja et al. (2011). Broz et al. (2010) analysed how the concentration of an ubiquitous and very broad group of plant secondary metabolites (plant phenolic compounds) depends on vegetation composition. Herbivory was mimicked by methyl jasmonate treatment of plants. They found higher levels of total phenolics in methyl jasmonate-induced *Centaurea maculosa* Lam. (Asteraceae) grown in monoculture than in plants grown with heterospecific neighbours. The authors concluded that the plant invests in defence when growing in monoculture since plants surrounded by conspecifics might suffer more from specialist herbivores (Root 1973) than plants surrounded by heterospecifics. The comparison of the relationship of plant secondary metabolite concentration and plant species richness in our study and the one by Broz et al. (2010) suggests that the biosynthesis of different plant metabolite types is affected differently by plant species richness. Furthermore, invasive species (like *C. maculosa* in the USA) may respond differently to heterospecifics than a native plant (as with *P. lanceolata* in our study).

Mraja et al. (2011) found a positive correlation between leaf catalpol concentrations of *P. lanceolata* and plant species richness in the surroundings, whereas a negative correlation was found between *P. lanceolata* aucubin concentrations and plant species richness. They performed their study within grassland plots where the plant species richness was experimentally kept constant. In contrast, our study was conducted in a ‘real world’ context with naturally varying environmental conditions (Fischer et al. 2010). These different methodological approaches applied to understand ecosystem functioning may explain the divergent outcomes (cf. Scherber et al. 2006; Unsicker et al. 2006).

Density of *P. lanceolata* plants did not correlate with IG concentrations in leaves, thus indicating that conspecific *P. lanceolata* neighbours did not affect concentrations of leaf IGs in our field study. In contrast, greenhouse experiments conducted by Barton and Bowers (2006) showed higher IG



concentrations in *P. lanceolata* grown together with conspecifics than in those grown without conspecific neighbours. This effect was especially marked 6 weeks after seedlings had been planted together, whereas 9 weeks later this effect decreased considerably. Since the plants we sampled were probably older than 9 weeks, we cannot discount that differences in IG concentrations might have occurred in younger plants due to different intraspecific competition levels. Many abiotic and biotic factors may impact on the metabolite content of a plant in the field (Barbosa et al. 2009); therefore, the effect of *P. lanceolata* conspecifics on IG concentrations that were found in the greenhouse (Barton and Bowers 2006) may be masked in the field by other factors.

### Plant metabolites and higher trophic levels

The abundances of the herbivores *M. pascuorum* and *M. labilis* did not correlate with the concentrations of plant IGs. Bernays and Graham (1988) suggest that secondary metabolites might not be the driving force in plant–herbivore interactions. Herbivores might also select their host plant in response to the top-down pressure exerted by predators and parasitoids (e.g. Randlkofer et al. 2007). Moreover, other toxic or anti-nutritive compounds, or primary metabolites, which were not considered in this study, might have affected abundances of the herbivores (Agrawal 2004).

While IG concentrations had no obvious impact on herbivore abundance, leaf N content correlated negatively with the abundances of both herbivores. Leaf N content was used as an indicator for the nutrient availability in the habitat and increased with escalating LUI. Furthermore, the LUI index correlated positively with several other plant response variables, i.e. Ellenberg N indicator values for the plant community, N and P concentration in plant biomass, P concentration in the soil, C/N ratio in the soil (Blüthgen et al. 2012); these correlations indicate greater availability of nutrients in plots with a more intense land use. Data analysis by SEMs also confirmed that in Hainich-Dün herbivore abundance patterns were influenced negatively by *P. lanceolata* leaf N content, whereas in the other regions no significant model could be obtained.

Thus, our data suggest that both herbivore species were more abundant in nutrient-poor habitats (here indicated by lower leaf N content). High N availability may lead to unbalanced leaf amino acid profiles or to higher organic acid concentrations in plants that could stress herbivores or may be even toxic for them (Brodbeck et al. 1990; Sarfraz et al. 2009b). Furthermore, fertilisation can lead to an imbalance in the protein/carbohydrate ratio because of an enhanced protein content (Cease et al. 2012). An experimental study performed in the same plots (Hancock et al.

2013) supports our findings on the negative correlation between N content in *P. lanceolata* and herbivore abundance since it showed that the weevils *M. pascuorum* and *M. labilis* occur in higher abundances in unfertilised than in fertilised experimental plots.

However, when considering the negative relationship between leaf N content and herbivore abundance found in our study, the weevil's feeding behaviour needs to be taken into account. The weevils studied here feed upon the plant stalks and leaves in the adult stage and live in the inflorescences during larval development. Since no data on the N contents of the plant parts fed upon by the larvae are available, we can only speculate how food primary metabolites (especially N supply) affected herbivore performance and abundance. A nutrient-rich habitat (as indicated by a high leaf N content) was shown to affect abundances of other herbivore competitors (Mattson 1980; White 1993; Throop and Ler dau 2004) and generalist insect enemies (Thompson 1999; Sarfraz et al. 2009a). Thus, a high N supply may also have negative, albeit indirect, effects on the abundance of the *Mecinus* weevils studied here. Further studies on the various factors that might be responsible for this negative correlation between leaf N content and herbivore abundance might help to elucidate the relevance of the *P. lanceolata* N content on herbivore abundance.

Parasitoid abundance was almost exclusively explained by host abundance across the three regions investigated and for Schorfheide-Chorin and Hainich-Dün when analysing the regions separately by SEMs. Parasitoids often respond to changes in host abundance with a Holling type II functional response (Holling 1959) according to which an increase in host abundance leads to enhanced parasitoid abundance. Furthermore, SEMs revealed a positive impact of IG concentrations on parasitoid abundance in the Schwäbische Alb. Many parasitoids are negatively affected by host plant secondary compounds (Ode 2006). The positive correlation of parasitoid abundance and plant IG concentrations might be explained by a possibly suppressive effect of IGs on host immune defences, as shown for the IG-sequestering butterfly species *Junonia coenia* (Smilanich et al. 2009). These effects might be direct ones or they might act indirectly by allocating energy from immune defence to metabolically costly detoxification (Bukovinszky et al. 2009; Smilanich et al. 2009). It is not known whether the specialist herbivores used in this experiment are able to sequester and store the IGs in their tissue (Baden et al. 2012). If so, a host insect endowed with sequestered plant defensive compounds might even shelter the developing endoparasitoid and protect it from hyperparasitoids or predators, thus finally increasing the parasitoid's success (Smilanich et al. 2009; Lampert et al. 2010).

The reason why the positive relationship between plant IG concentrations and parasitoid abundance was only

detected in the Schwäbische Alb region, but not in the two other, may be region specificity of the pattern of abiotic and biotic factors (see “Materials and methods”). For example, the (a)biotic factors present in Hainich-Dün and Schorfheide-Chorin might have resulted in higher herbivore abundance, which in turn might conceal a positive link between IG concentration and parasitoid abundance as detected by an explanatory model for the Schwäbische Alb.

## Conclusion

Our field study showed several correlations between vegetation composition, host plant chemistry and the abundance of herbivores and their parasitoid in an ubiquitous tritrophic system occurring in real landscapes. These correlations give rise to some hypotheses that need to be tested now with specific experiments. The detected correlations suggest that plant species richness can affect the concentration of secondary metabolites in *P. lanceolata* and that these effects on the host plant can influence higher trophic levels in a human-altered landscape; however, the correlations further indicate that these changes do not have the same effects on abundances of members from the second and third trophic level. The regional differences in land use and the effects observed on the different trophic levels emphasise the necessity of considering regional characteristics when approving management strategies for maintenance of multitrophic relationships and ecosystem functions in grasslands in a human-altered landscape.

**Author contribution statement** T. M. and E. O. conceived and designed the study. N. W. and C. H. collected field data. N. W. collected chemical data and analysed all data. N. W., M. H., and T. M. wrote the manuscript; other authors provided editorial advice.

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