

Ecography

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Supplementary material

Appendix 1.

Supplementary methods

Species sampling

In order to cover as many organism groups as possible, we used a diverse set of sampling methods and techniques on all 27 study plots (see also Schuldt et al. 2015, Schuldt et al. 2017). Species richness and individual number (i.e. abundance) of all woody plants was measured by a complete inventory of all trees and shrubs taller than 1 m height when initially establishing the plots in 2008 (Bruehlheide et al. 2011). Simultaneously, the species richness and abundance of all herbaceous plants (i.e. non woody individuals <1 m height) was assessed in the central 10 m × 10 m area of each plot (Both et al. 2011).

To quantitatively sample arthropods, the by far most abundant and diverse group of macroorganisms in tropical and subtropical forests (e.g. Basset et al. 2012), several different techniques were used. Most sampling methods yielded many more taxonomic groups than the ones further identified by us. For reasons of logistics, feasibility and availability of taxonomic expertise, not all collected taxa could be included into this study. Per plot, 4 pitfall traps (diameter 8.5 cm, height 15 cm) were placed at the corners of the central 10 m × 10 m plot from March to September 2009. Traps were filled with a preserving solution (40% ethanol, 30% water, 20% glycerol, 10% acetic acid, few drops of detergent) and emptied biweekly (Schuldt et al. 2011, Staab et al. 2014b). All Araneae, Chilopoda, Curculionidae, Diplopoda and Formicidae were sorted out from the total pitfall catches and further identified (see

below). From May to August 2010, 4 flight interception traps were operated per plot. Those traps consisted of two crossed plexiglass rectangles (50 cm × 30 cm) covered by a lid and placed above a funnel, to which a replaceable PE bottle (500 ml) filled with preserving solution (see pitfall traps) was attached. From the total flight interception catches, all Cerambycidae, Formicidae and Scolytidae were sorted out and further identified (see below). In fall 2011 and spring 2012, 25 trees and shrubs in the understory were sampled 3 times each (total of 75 samples per plot) with beating. For this, a funnel-shaped cloth sheet was placed under the plant individual, which was quickly hit 7 times with a beating stick to shake arthropods onto the sheet, where all individuals were collected with forceps and aspirators (Schuldt et al. 2014). From the total catches, all Araneae, Formicidae and larval Lepidoptera were taken. To collect cavity-nesting predatory wasps and their parasitoids, reed-filled trap nests were exposed from September 2011 to October 2012. Trap nests were checked monthly and occupied nests were collected and reared in the laboratory until specimens had hatched (Staab et al. 2014a). Finally, ants were also collected with protein (canned fish) and carbohydrate (honey water) baits in May 2012. In all nine 10 m × 10 m subplots inside each plot, 2 bait pairs (each at the ground and at breast height, i.e. 36 baits per plot) were exposed for 3 hours and all ants interacting with baits were collected (Schuldt and Staab 2015).

Arthropods in each focal taxonomic group were sorted to morphospecies by comparing external morphological characters, e.g. genitalia for spiders. Whenever possible, we further identified to species level, such as for most Formicidae. Individuals of each species and morphospecies were counted to yield a measure of species abundance. We are aware that using raw abundance data (individual numbers) might be problematic for ants, which have colonial life style, albeit with very large differences in colony size and foraging distances among species. Thus, it might sometimes be more suitable to use ant abundance measures based on the occurrence/incidence of ant colonies than to use individual numbers. However, when using occurrences it would not be possible to combine ant data with the abundance of

other predators, which are not constrained by living in colonies. Thus, we could not analyse trophic guilds that are a central scheme of our study, and we decided to use ant individual numbers as measure of ant abundance. Data for each considered group collected with the different sampling methods were pooled per plot, as we were interested in the effects of elevation and associated environmental variables at the community level.

To sample microorganisms (bacteria and fungi) DNA was extracted from soil samples and sequenced. Per plot, 8 soil cores were collected in September 2012. The top 0-10 cm of each core was sieved to remove coarse material, and freeze dried to prevent degradation of DNA. Two samples each were combined to 4 composite samples (comparable to the 4 pitfall and 4 flight interceptions traps per plot), microbial DNA was extracted from 1 g (dry mass) of each composite soil sample with MoBio soil DNA extraction kits (Mo Bio Laboratories, Carlsbad, CA, USA), and extracted DNA was stored at -20°C until further processing. To obtain sufficient DNA quantities for pyrosequencing, bacterial and fungal DNA was amplified using custom fusion primers. For bacteria, we used the primers BAC 341F and BAC 907R that amplify the V3-V5 region of the bacterial 16S rRNA gene (Muyzer et al. 1995, Muyzer and Smalla 1998). For fungi, the primers were ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) to amplify the fungal internal transcribed spacer (ITS) rRNA region. PCR conditions followed Wubet et al. (2012). PCR products were purified with QIAquick Gel Extraction Kits (QIAGEN, Venlo, Netherlands). Equimolar mixtures of each amplicon library were pyrosequenced unidirectionally from the 907R and ITS4 ends with 454 Titanium amplicon sequencing kits on a 454 Genome Sequencer FLX + System (Roche Applied Biosystems, Basel, Switzerland).

Sequence reads were filtered and normalized to 10,000 fungal ITS and 20,000 bacterial 16S rDNA reads per plot (MOTHUR, Schloss et al. 2009) in order to allow comparison among plots. Species level molecular operational taxonomic units (OTUs) were delineated with a 97% similarity criterion (see e.g. Wubet et al. 2012). The taxonomy of

bacterial and fungal OTUs was assigned with the databases SILVA SSU (Quast et al. 2013) and UNITE (Kõljalg et al. 2005), respectively. OTUs belonging to non-target taxa were removed. OTUs occurring as singletons, doubletons and tripletons have a high probability to be results of sequencing errors (Kunin et al. 2010) and were consequently removed from the data. For bacteria and fungi, we did not score abundances as data obtained from sequence reads cannot directly be interpreted as individual numbers, which was the measure of abundance for all other organism groups.

Trophic guilds

Grouping organisms from different taxa to functional groups or trophic guilds allows meaningful analyses (Simberloff and Dayan 1991) of how biotic and abiotic habitat properties influence organisms sharing a common trophic ecology, and is commonly used in biodiversity studies, particularly in diverse ecosystems (e.g. Basset et al. 2012). For our study, we grouped all higher primary producers (i.e. woody and herbaceous plants) to the trophic guild ‘plants’. As ‘herbivores’, we grouped Lepidoptera and Curculionidae, which were from all arthropod taxa identified to species or morphospecies the taxa of which most species feed primarily on green plant material. In turn, the arthropods with a detritivorous, xylophagous and saproxylophagous diet (Cerambycidae, Diplopoda, Scolytidae) were grouped as ‘detritivores’. Arthropods known to at least partially hunt other arthropods to prey on them were grouped as ‘predators’. From the identified taxa these were Araneae, Chilopoda, Formicidae, cavity-nesting wasps and their parasitoids.

Among the multitude of bacterial lineages (see Schuldt et al. 2015), the eight phyla Acido-, Actino- Alphaproteo-, Betaproteo-, Gammaproteo-, Deltaproteobacteria, Chloroflexi and Bacteroidetes accounted for >93% of all bacterial DNA sequence reads and were grouped as ‘bacteria’. We are aware that those bacterial lineages likely contain species with various trophic ecologies, making the exact determination of functional groups in bacteria difficult.

Likewise, the single fungal lineages (Agaricomycetes, Arachaeorhizimycetes, Dothideomycetes, Eurotiomycetes, Glomeromycetes, Leotiomycetes, Orbiliomycetes, Sordariomycetes, Tremellomycetes, Wallemiomycetes, Zygomycota), which contain arbuscular and ectomycorrhiza, saprophytic and pathogenic fungi were grouped to the guild 'fungi'.

Environmental variables

For every plot, we measured a broad range of environmental variables that are known to be associated to elevation. Temperature was measured every 30 min from July 2011 to June 2012 with data loggers (HOBO U23 Pro v2, Onset Computer Corporation, Cape Cod, MA, USA) installed ca. 30 cm above the ground. Mean annual temperature was calculated from the individual measurements. The same digital elevation model (SRTM v4, Jarvis et al. 2008) used for plot elevation was also used to calculate the available land area of different elevation bands. For this, elevation steps of 50 m were used in a range from 200 to 900 m a.s.l. (i.e. 200-250 m, 250-300 m, etc.) within a 20 km radius from the geographic centre of the GNNR. This radius was appropriate because the relative available area per band did not change with larger radii (e.g. 50 km).

Soil properties such as pH and C:N-ratio do not generally depend on elevation, but can locally be correlated with elevation (Smith et al. 2002). To determine soil pH, we collected nine independent top-soil samples (0-10 cm depth) per plot in 2009, measured the pH in 1 M KCl solution and calculated a mean pH value. From the same soil samples we determined soil carbon (C) and nitrogen (N) content (measured with a Vario ELIII elemental analyzer, Elementar, Hanau, Germany), and calculated the soil C:N ratio.

The age of the secondary forest was determined by combining tree diameter measurements with counting tree rings from stem core drillings of the largest trees in a given plot. The age of the 5th largest tree was taken as plot-specific successional age. This measure

proved to be representative (Bruelheide et al. 2011), as in local land use practices single large trees were frequently kept to provide shading and those trees may still be present now.

The dimensions and the species identity of each deadwood piece were determined during an inventory in winter 2008/2009 to calculate deadwood mass. In the entire plot, items with a diameter $> 10\text{cm}$ were recorded; in the central $10\text{ m} \times 10\text{ m}$, all items $>3\text{ cm}$ were additionally recorded. The mass of stem segments, branches, and stubs was calculated by multiplying the sample volume with the species-specific density. The mass of whole trees was calculated with allometric equations for trees in the GNNR (Brezzi 2015). Deadwood mass per plot was obtained by summing the mass of all woody debris.

Finally, the aspect of each plot was determined. Mathematically, aspect is a circular variable represented by the compass direction of a slope. For linear statistics, we used the sine and cosine transformation of the aspect expressed by eastness and northness (Beers et al. 1966).

Statistics

Before calculating the multiple linear models, all response variables (species richness and abundance of trophic guilds) were scaled between 0 and 1 by dividing the plot-specific values through the maximum value of the respective trophic guild, in order to allow comparison of correlations. The full set of environmental variables considered as fixed effects in those models consisted of MAT, MAA, deadwood mass, tree age, soil pH, soil C:N ratio, eastness and northness, and those variables were standardized (mean=0, SD= ± 1).

Full models of each trophic guild contained slightly different sets of predictor variables, because the model structure was each based on general ecological relationships and a priori considerations of correlations between elevation, species richness/abundance and environmental variables. Before fitting the full models, collinearity among all environmental variables was assessed. In case two environmental variables were correlated with Spearman's

$\rho > 0.7$ (Dormann et al. 2013), we retained the variable that is, in our opinion, ecologically more directly interpretable (see Table A1). This applied to the pairs of collinear environmental variables MAT vs. MAA as well as deadwood vs. tree age (except in the reduced detritivore and fungi data set). Likewise, soil C, soil N and soil C:N were collinear and thus soil C:N as the variable combining information on soil C and soil N was selected.

All full models included tree age and the aspect components. For predators, herbivores, plants, bacteria and fungi, MAT was included, which is expected to have a stronger effect on their richness than MAA, as temperature immediately affects the metabolic rate of small ectotherms, photosynthetic organisms and microorganisms. Further, for microorganisms much smaller spatial scales than represented in 50 m elevation bands may be relevant. The full herbivore model did not include any further variables. The full detritivore model included neither MAT nor MAA. Both variables strongly decreased with elevation, as expected (McCain & Grytnes 2010). Detritivore richness, in contrast, increased with elevation. Thus, MAT and MAA would be statistically negatively related to this particular trophic guild, which contradicts established theoretical considerations (McCain & Grytnes 2010). Instead, as we had early indications that detritivore richness might be primarily driven by deadwood mass along the elevation gradient, this variable was included in the full model for this trophic guild. As deadwood can also serve as a food resource for soil fungi, the full fungi model likewise included deadwood mass. Additionally, the full models of fungi, bacteria and plants included soil pH and C:N ratio. Full abundance models had for each trophic guild the same structure as the corresponding richness model.

The initial full models were simplified with the function ‘dredge’ of the R-package ‘MuMIn’ (Barton 2013). This model selection procedure calculates all possible candidate models with the fixed effects of the respective full model and orders the models by Akaike weights, based on the Akaike Information Criterion corrected for small sample sizes (AICc), to find a minimal model with lowest AICc (Burnham et al. 2011). If two candidate models

were equally likely ($\Delta\text{AICc}<1$), the more parsimonious model, i.e. with the lower number of fixed effects, was chosen. Due to the mathematical formulation of AICc , relative differences in AICc depend on sample size. We thus prefer the more conservative $\Delta\text{AICc}<1$ over the frequently used $\Delta\text{AICc}<2$ because our sample size of 27 plots was relatively small.

The structure of the guild-specific path analyses (PA) was determined by the outcome of the most parsimonious multiple linear models for species richness. For predators, detritivores, herbivores and plants, all selected environmental variables had direct paths on species richness as well as indirect paths via species abundance. Guild-specific variables included in the detritivore PA were tree age, deadwood mass and eastness. In the detritivore PA (as opposed to all other trophic guilds), we did not allow direct paths from MAT and MAA to detritivore richness and abundance for reasons explained above. In contrast, we included the direct path from elevation to deadwood mass and the corresponding indirect paths via tree age. The fungi PA included tree age and both aspect components. Paths of northness on plant species richness and abundance was added to the plant PA, following the minimal plant model. Finally, the PAs for predators, herbivores and bacteria, contained only the indirect paths of elevation via MAT and MAA, and of both variables via abundance.

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Supplementary tables

Table A1 Overview of environmental variables included in the full richness (R) and abundance (A) models of trophic guilds. Grey shaded cells indicate variables included in individual minimal richness (R) or abundance (A) models. (- = not included into the full model due to a priori considerations [see main text and supplementary methods]; Ex = excluded from both, richness and abundance models, during model selection; C (x) = collinear with variable x and not included in the full model in favour of variable x).

<i>Trophic guild</i>	<i>Variables included in minimal models</i>					<i>Variables not included in minimal models</i>		
	log (DM)	MAT	Tree age	Northness	Eastness	MAA	Soil pH	Soil C:N ratio
Predators	-	R+A	Ex	Ex	Ex	C (MAT)	-	-
Detritivores ¹	R+A	-	R	Ex	R	-	-	-
Herbivores	-	Ex	Ex	Ex	Ex	C (MAT)	-	-
Plants	-	Ex	A	R	A	C (MAT)	Ex	Ex
Bacteria	-	Ex	Ex	Ex	Ex	C (MAT)	Ex	Ex
Fungi ¹	Ex	Ex	R	R	R	C (MAT)	Ex	Ex

DM: deadwood mass, MAT: mean annual temperature, MAA: mean available area.

¹ Based on a dataset from which three plots were removed (for details see Materials and Methods).

Table A2 Results of linear regressions showing the correlation between elevation and the species richness of individual organism groups.

Taxon	Trophic guild	Slope ± SE	Intercept ± SE	<i>t</i>	<i>p</i>	<i>R</i>²
Acidobacteria ¹	bacteria	-0.009 ± 0.019	312.201 ± 10.430	-0.466	0.646	0.010
Actinobacteria ¹	bacteria	-0.011 ± 0.011	137.922 ± 6.282	-0.956	0.349	0.040
Alphaproteobacteria ¹	bacteria	0.026 ± 0.028	401.753 ± 15.702	0.923	0.366	0.037
Bacterioidetes	bacteria	0.004 ± 0.011	78.388 ± 6.291	0.367	0.717	0.005
Betaproteobacteria	bacteria	0.002 ± 0.007	58.344 ± 3.880	0.346	0.732	0.005
Chloroflexi	bacteria	-0.004 ± 0.018	188.496 ± 9.927	-0.224	0.825	0.002
Deltaproteobacteria	bacteria	0.036 ± 0.016	122.732 ± 9.204	2.219	0.036	0.165
Gammaproteobacteria	bacteria	0.036 ± 0.018	129.318 ± 10.223	2.014	0.055	0.140
Cerambycidae ¹	detritivore	0.019 ± 0.005	-1.531 ± 2.538	4.242	<0.001	0.450
Diplopoda ¹	detritivore	0.005 ± 0.002	-0.104 ± 1.200	2.537	0.019	0.226
Scolytidae ¹	detritivore	0.013 ± 0.005	14.873 ± 2.856	2.609	0.016	0.236
Arbuscular mycorrhizae	fungi	0.009 ± 0.004	4.38 ± 2.236	2.369	0.026	0.183
Ectomycorrhizae ¹	fungi	0.008 ± 0.012	94.210 ± 6.685	0.701	0.491	0.022
Pathogenic fungi	fungi	-0.001 ± 0.005	20.631 ± 2.870	-0.203	0.841	0.002
Saprophytic fungi ¹	fungi	0.042 ± 0.026	185.558 ± 14.307	1.649	0.113	0.110
Lepidoptera	herbivore	-0.003 ± 0.004	12.4 ± 2.226	-0.658	0.517	0.017
Curculionidae	herbivore	0.005 ± 0.004	3.792 ± 1.979	1.402	0.173	0.073
Herbaceous plants	plant	0.005 ± 0.007	4.803 ± 3.710	0.734	0.470	0.021
Woody plants	plant	0.008 ± 0.012	37.378 ± 6.868	0.676	0.505	0.018
Araneae	predator	-0.013 ± 0.007	52.389 ± 3.994	-1.848	0.076	0.120
Chilopoda	predator	-0.002 ± 0.002	6.733 ± 1.399	-0.659	0.516	0.017
Formicidae	predator	-0.012 ± 0.004	34.393 ± 2.263	-2.920	0.007	0.254
Hymenoptera parasitoid	predator	-0.004 ± 0.002	4.743 ± 1.331	-1.515	0.142	0.084
Hymenoptera predator	predator	0.001 ± 0.001	2.244 ± 0.781	1.061	0.299	0.043

Significant *p*-values (*p*<0.05) are indicated in bold.

¹ Based on a dataset from which three plots were removed (for details see Materials and Methods).

Table A3 Results of linear regressions showing the correlation between species richness and log-transformed abundance of trophic guilds.

Trophic guild	Slope ± SE	Intercept ± SE	<i>t</i>	<i>p</i>	<i>R</i> ²
Predators	14.253 ± 4.743	-15.513 ± 33.513	3.005	0.006	0.236
Detritivores ¹	7.977 ± 1.207	-14.786 ± 7.393	6.610	<0.001	0.650
Herbivores	0.598 ± 1.048	15.202 ± 3.990	0.571	0.573	0.013
Plants	1.983 ± 3.606	35.859 ± 24.406	0.550	0.587	0.012

Significant *p*-values (*p*<0.05) are indicated in bold.

¹ Based on a dataset from which three plots were removed (for details see Materials and Methods).

Supplementary figures



Figure A1 Photograph showing the typical landscape in the study site, the Gutianshan National Nature Reserve. Although mountains are relatively low and completely forested, the rough topography and rapid changes in elevation are dominant features of the landscape. The photograph was taken facing northwards at $29^{\circ}14'28''$ N / $118^{\circ}07'14''$ E and at an elevation of about 600 m a.s.l. by Michael Staab on 09-Oct-2012.

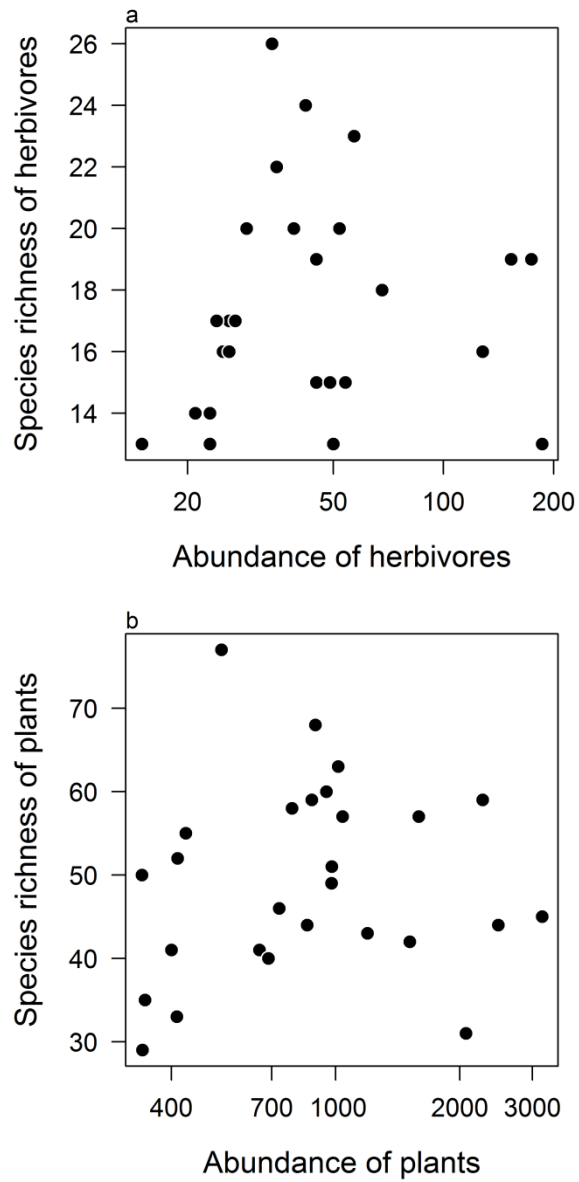


Figure A2 Relationships between species richness and species abundance for herbivores (a) and plants (b). Statistical details are shown in Table A3. Note that all x-axes are log-scaled.

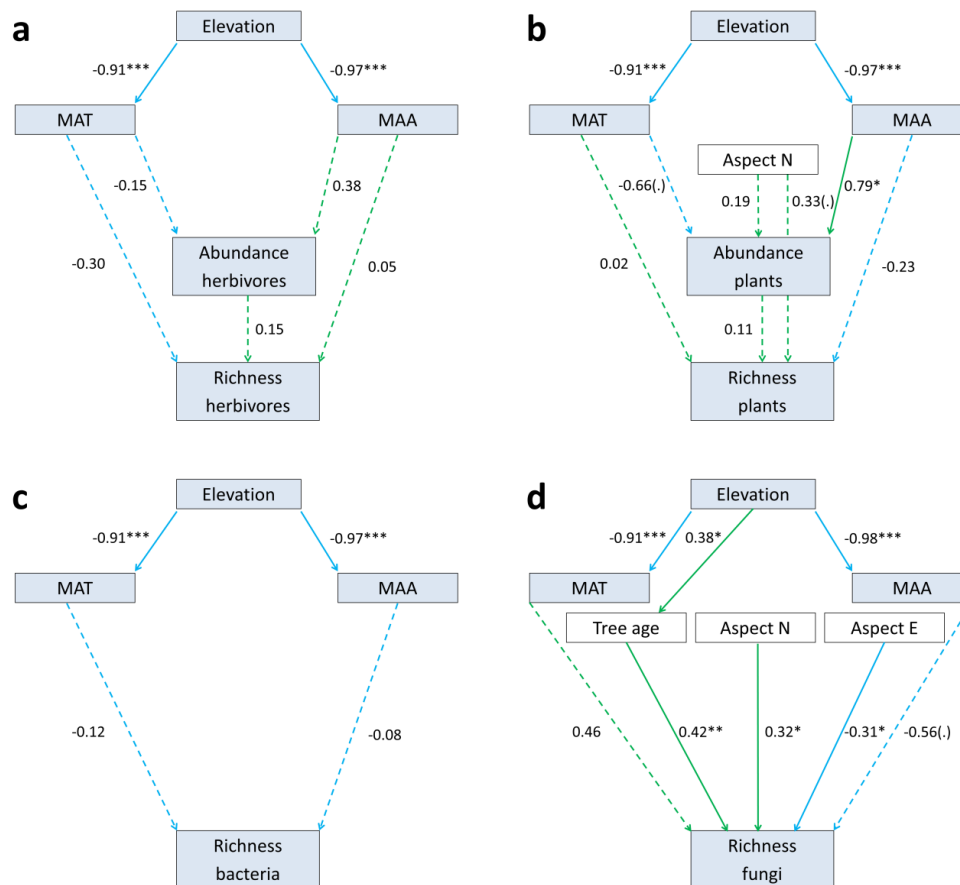


Figure A3 Path models showing how elevation affects species richness of herbivores (a) and plants (b) directly via mean annual temperature (MAT), mean available area (MAA), and other environmental variables, or indirectly via the effects of the environment on abundance. Path models for bacteria (c) and fungi (d) richness are also shown. Numbers next to arrows give the standardized path coefficients. Significant causal paths are indicated with full arrows, non-significant paths with dashed arrows. Green arrows indicate positive relationships, blue arrows negative relationships. Guild-specific predictor variables are shown in unfilled boxes. Path model (d) is based on a reduced data set excluding three plots (for details see Materials and Methods). Significances were obtained from z-tests and are *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and (.) $p < 0.1$.