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Plant diversity effects on crop yield, pathogen incidence, and secondary metabolism on cacao farms in Peruvian Amazonia

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ABSTRACT

Biodiversity may be positively related to crop yield, but the mechanisms by which such effects are realized are as yet poorly understood. Reduced pest incidence may be one cause. To better predict the quality and strength of biodiversity effects in cacao agroforestry systems and to disentangle potential drivers, we analyzed relationships of plant diversity with crop quantity (yield, fruit set, fruit size), pathogen incidence (*Moniliophthora perniciosa*,*Moniliophthora roreri*, *Phytophthora* spp.), and with the profile of selected secondary compounds (methylxanthines and polyphenols) in seeds of 48 cacao trees cultivated on 14 farms in Peruvian Amazonia. Our results revealed no correlation of yield per hectare or total fruit set with plant alpha diversity measures on the studied cacao farms. However, the number and size of ripe fruits without fungal infestation increased at higher diversity of the herb and shrub layer and at lower diversity and smaller basal area of shade trees. Greater diversity in the herb and shrub layer reduced the incidence of the *Phytophthora* pathogen but increased the incidence of *M. roreri*. At higher alpha diversity in the understory, contents of caffeine, theobromine, and catechin hydrate in cacao seeds significantly increased. The changes in plant secondary compounds showed inconsistent relations with the infestation rates of fungal pathogens. While trees infested with *M. perniciosa* showed higher contents of polyphenols and caffeine in seeds, cacao trees with higher caffeine content in seeds were less likely to be affected by *Phytophthora*. Similarly, a higher epicatechin content in seeds was associated with reduced *M. roreri* incidence. Our data provide evidence for a tight interplay of biodiversity, pathogen incidence, and the crop's secondary metabolism on cacao farms. Overall, considering biochemical traits in yield-diversity relationships allowed for a better understanding of the contribution of biotic interactions to biodiversity effects in tropical agroforestry systems.

1. Introduction

Agrarian ecosystems, which cover about 30% of the Earth's ice-free terrestrial surface (Hurtt et al., 2011), play a pivotal role in biodiversity conservation worldwide (Fahrig et al., 2011). Although there is growing evidence that biodiversity may be beneficial to agricultural production (e.g., Bonin and Tracy, 2012; Hooper et al., 2005; Letourneau et al., 2011; Sirrine et al., 2008; Szumigalski and Van Acker, 2006), creating highly productive agroecosystems with a high diversity (i.e., land sharing according to Phalan et al., 2011) remains challenging—mainly because the mechanisms through which yield and biodiversity interact are as yet poorly understood. In particular, the competitive and complementary interactions among plants as well as the indirect effects mediated by soil microorganisms (Chung et al., 2007; Zak et al., 2003), pathogens (Gosme et al., 2012; Hol et al., 2013; Keesing et al., 2010), or arthropods (Bartomeus et al., 2014; Gosme et al., 2012; Groeneveld et al., 2010; Wielgoss et al., 2014) make it difficult to predict net biodiversity outcomes with confidence (Letourneau et al., 2011). Consequently, the scientific literature reveals negative (Clough et al., 2011), positive (Cierjacks et al., 2016; Hooper et al., 2005; Kamoshita et al., 2014; Samedani et al., 2014) or missing correlations (Clough et al., 2011; Pollnac et al., 2009) between plant diversity and crop yield. Moreover, when the biomass rather than the diversity of coexisting plants is considered, opposing,

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often negative effects on yield are found (Cierjacks et al., 2016; Pollnac et al., 2009).

Tropical agroforestry systems such as cacao farms have been frequently highlighted as good examples of both highly diverse and productive agroecosystems (e.g., Daghela Bisseleua and Vidal, 2008; Jagoret et al., 2011; Ofori-Bah and Asafu-Adjaye, 2011; Rice and Greenberg, 2000; Schroth et al., 2011; Van Bael et al., 2007). As the species in agroforestry systems show high functional diversity in terms of resource acquisition, complementary interactions among plants seem more common than competitive ones (Schwendenmann et al., 2010; Smith et al., 2010). Accordingly, adding functionally different crop species such as banana to cacao tree plantings resulted in higher cocoa yield overall and per plant (Deheuvels et al., 2012; see also Ofori-Bah and Asafu-Adjaye, 2011) and in greater robustness against pests (Sabatier et al., 2013).

In addition to quantitative measures such as crop yield, qualitative traits of crops are increasingly taken into consideration for assessing the success in agriculture (Bartomeus et al., 2014), particularly in the case of gourmet foods such as cocoa (e.g., Cooper et al., 2008; Marcano et al., 2009). The quality of unfermented cacao seeds is amongst others determined by the content of methylxanthines such as caffeine and theobromine (Brunetto et al., 2007) as well as the appropriate content of phenolic compounds (e.g., catechin and epicatechin; Lieberei and Reisdorff, 2012). These substances of the plant's secondary metabolism also play a relevant role in the crop's resistance against pathogens (Marcano et al., 2009) of which witches' broom (*Moniliophthora perniciosa*), frosty pod (*Moniliophthora roreri*), and black pod (*Phytophthora* spp.) are among the most economically important such fungi (e.g., Acebo-Guerrero et al., 2012; Krauss and Soberanis, 2002; Soberanis et al., 1999; Somarriba and Beer, 2011). The inclusion of these quality traits in our study may reveal novel insights into the mechanisms of biodiversity effects as, in addition to yield, pathogen incidence and the physiological reaction in terms of plant secondary compounds can be assessed at different diversity levels.

Peruvian Amazonia is particularly suited for a study on cacao agroforestry systems due to the well-documented plant material covering both the cacao clone CCN-51 and local varieties. The cacao tree (*Theobroma cacao* L.) originates from the Amazonian tropical lowland rainforests (Lieberei and Reisdorff, 2012), which is the reason why a high genetic diversity of cacao can be found there. However due to the prevalent coca cultivation during the past decades in many regions of Peru, cacao cultivation was of minor importance. Since about ten years, the cultivation of coca has again been replaced by cacao farming but, instead of making use of the local varieties with higher levels of genetic variation, the clone CCN-51 is currently the most abundant one (García Carrión, 2012). It has been planted over huge areas due to high yields and pronounced resistance against pathogens. This clone is of rather moderate quality in terms of taste and aroma and yields a product that is classified as "bulk cocoa" (García Carrión, 2012). Some farmers have maintained local cacao varieties with pronounced plasticity in the quality traits (García Carrión, 2012). Some of these yield a higher grade of cocoa, known as "fine and flavor cocoa", due to specific aroma notes and may command a price above stock market value.

Traditionally, cacao is planted in agroforestry systems with shade trees. In contrast, high-performance clones like the CCN-51 are often grown as full-sun plantations in order to gain the highest possible yields. The farming conditions in the study area range from intense full-sun fields to traditional mixed agroforestry cultivation which implies different biodiversity levels that – in addition to the crop's genetic background – may translate to changes in pathogen resistance and the contents of plant secondary compounds.

This study analyzes possible biodiversity effects and interactions with farm management and soil conditions on cocoa yield, pathogen incidence, and plant secondary compounds such as polyphenols and methylxanthines. In particular, we considered the following questions: (1) Do biodiversity of the herb and shrub layer and of the shade tree layer as well as biomass of the respective vegetation layers influence cocoa yield, pathogen incidence, and the polyphenol and methylxanthine content of seeds? (2) How do farm management parameters (pH, electrical conductivity and use of fertilizers and pesticides) affect the relationship of biodiversity with crop yield and quality? (3) Are possible biodiversity effects related to the cacao varieties used (low genetic variability in CCN-51 vs. high variability in local varieties)? Based on the results, practical recommendations for optimizing biodiversity conservation, yield, and product quality will be given, which may be generalized to other tropical agroforestry systems.

2. Materials and methods

2.1. Study area

The study was carried out in the Tocache province in the department of San Martín, northeastern Peru (8°11′19.565″S, 76°33′31.626″W; see Supplementary data, interactive map). Until recently the area was widely used for illicit coca cultivation. To provide the farmers with a legal land-use alternative, the United Nations Office on Drugs and Crime (UNODC) implemented a project in the regions of San Martín, Huanuco, and Ucayali to replace coca with cacao. In parallel, already existing cacao farms were supported by technical assistance of the UNODC in order to maintain the exsisting plant material. For novel cacao plantings initially CCN-51 was the preferred breeding line owing to its high performance and pest resistance. The next phases involved cultivating international fine and flavor clones to provide farmers with an income above the stock market price. In parallel, the farmers were encouraged to maintain local cacao varieties with above-average yield, little susceptibility to pests and diseases, and pronounced flavor potential.

In the past, the area had been nearly entirely covered by tropical lowland, sub-montane, and montane rainforests which were largely converted by slash-and-burn activities to crop fields, pastures, and agroforestry systems. The main soil types in the area are Inceptisols (Dystrudepts; Programa de Desarollo Alternativo Tocache Uchiza, 2008). The climate is typical for tropical lowlands (Soberanis et al., 1999). Mean annual precipitation at Tocache Nuevo, which sits in the Huallaga River valley in the eastern foothills of the Andes mountains (497 m asl.), is 2400 mm with a pronounced rainy season between October and March. Mean annual temperature is 24.7 °C, and mean relative humidity is 70–80% (Programa de Desarollo Alternativo Tocache Uchiza, 2008; Soberanis et al., 1999).

2.2. Study design

In the study area, we randomly selected 14 cacao farms in June 2014. Total farm sizes ranged between 2 and 15 ha with an average of 4.92 ha. Most farms (11) were exclusively used for cacao cultivation with a mean area of 3.23 ha. All farm owners (12 males and 2 females) except one were associated in a cacao cooperative. Cacao trees were mainly planted in squares or quincunx of 3 m × 3 m. On each farm, pairs of trees – one CCN-51 individual and another one from a local variety – were selected. To guarantee similar site conditions for the two trees, the maximum distance between them was 15 m. In cases where more than one suitable local variety was present, additional pairs were selected at least 100 m away. Overall, this approach resulted in 48 cacao trees in 24

pairs located on 14 farms. Each tree was the center of a study plot for analyses of site conditions concerning biodiversity, pathogen incidence, plant secondary compounds in seeds, soil parameters, and shading.

Shade management within the cacao farms is based on different tree species many of them characterized by additional economic values as fruit-trees, timber or firewood (see Supplementary data, Annex 1).

2.3. Field data collection

The species diversity of the herb and shrub layer was assessed within a squared plot of 5 m × 5 m around each study tree by carrying out a vegetation relevé according to the methodology of Braun-Blanquet (1964). Plant determination was conducted at Universidad Nacional Agraria de la Selva (UNAS), Tingo María, Peru. The diversity of shade trees (all individuals >4 m) was recorded on a 20 m × 20 m plot with the study trees as center. Within each plot, we counted and identified all shade trees and measured their circumference at breast height. Identification of trees was done in the field and supported by local agrarian specialists of UNODC, Peru. The plot's total basal area of all shade trees was used as a proxy for aboveground biomass in this vegetation layer.

Four 100-cm^3 soil samples (4 cm in depth) were collected in all cardinal directions adjacent to the study trees and pooled.

Each study cacao tree was characterized in terms of its circumference at breast height and pathogen incidence. For *Phytophthora* spp. and *M. roreri* we counted infested pods, and for *M. perniciosa* the absolute number of deformed twigs was recorded. In addition, all ripe and developing fruits per tree with a length of minimum 8 cm were counted to calculate fruit set and the proportion of infested pods per tree. At least three ripe, healthy fruits of each tree were sampled. We measured length and circumference of the pods. For biochemical analyses, unfermented cacao seeds were removed from the pods and air-dried.

Insolation in percent was estimated visually on-site, and light incidence at the cacao tree and at the herb layer within the 25-m^2 plots was assessed separately.

Farm management was evaluated based on interviews with the farm owners who were asked about mean yield per hectare and year, the use of fertilizers and pesticides, the age of planted cacao trees and pruning activities (Table 1) along with general socioeconomic information. All management parameters reflect the most frequently applied practices during the last three years and refer to the entire farms without differentiation into the genetic background of the study trees.

2.4. Lab analyses

2.4.1. Chemicals and standards

Unless otherwise specified, all chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). Epicatechin, catechin hydrate, cyanidine-3-arabinosid, cyanidine-3-galactosid, theobromine, and caffeine were purchased from Sigma. Water was deionized in an Elga water purification system (PURELAB Option, Elga, UK).

2.4.2. Degreasing of cacao seeds

Seed samples of each cacao tree were dried for 48 h at 40 °C, and subsequently, seed shells were removed. For degreasing, 2 g of each sample were milled to a powder with a particle size of approx. 1 μm in a ball mill (MM200, Retsch, Haan, Germany) with 10 mL *n*-hexane at a frequency of 20 s^{-1} for 10 min. Grist was rinsed three times with 25 mL petroleum ether (boiling range 40–60 °C) using a

Table 1
Farm management and mean soil parameters of all farms analyzed. Values in bracket: standard error.

Farm	Number of plot pairs	Yield (kg ha^{-1} $year^{-1}$)	Fertilizer use	Pesticide use	Sanitary pruning per year	Shape pruning per year	Tree age (year)	Soil				
								C content (%)	N content (%)	C/N ratio	pH (H_2O)	Electrical conductivity (μS/cm)
1a	1	2300	–	–	5	4	12	2.8 (0.3)	0.27 (0.03)	10.4 (0.0)	5.8 (0.3)	162.8 (37.9)
1b	2	2000	Organic	–	0	7	8	3.0 (0.2)	0.30 (0.02)	10.1 (0.2)	5.2 (0.0)	198.4 (43.7)
2	1	1500	Organic	–	5	1	20	2.7 (0.7)	0.25 (0.05)	10.9 (0.8)	5.8 (0.2)	154.0 (13.2)
3	1	1500	Organic	–	5	1	14	2.4 (0.1)	0.26 (0.02)	9.1 (0.0)	5.8 (0.2)	144.7 (1.3)
4	2	1000	Organic	Organic	5	1	7	2.2 (0.2)	0.23 (0.03)	9.6 (0.2)	5.2 (0.1)	34.5 (29.9)
5	2	1500	Organic	Organic/ chemical	5	1	4	4.3 (0.3)	0.42 (0.02)	10.2 (0.2)	5.4 (0.0)	7.1 (1.2)
6	1	2000	Organic	Organic	5	1	12 (4)	3.7 (0.3)	0.35 (0.02)	10.3 (0.5)	6.7 (0.0)	93.7 (81.8)
7	1	2000	–	Chemical	5	3	17 (1)	2.9 (0.4)	0.29 (0.03)	9.9 (0.2)	5.8 (0.4)	74.7 (71.3)
8	3	2500	Organic/ chemical	Organic	0	4	9 (1)	3.3 (0.2)	0.31 (0.01)	10.7 (0.2)	6.1 (0.1)	129.2 (56.4)
9	1	1800	–	–	0	3	11 (3)	4.0 (0.2)	0.36 (0.01)	11.2 (0.1)	6.4 (0.1)	4.2 (0.8)
10	1	1800	Organic	Organic	5	4	5	5.6 (1.5)	0.45 (0.11)	12.2 (0.5)	6.7 (0.1)	270.6 (40.5)
11	1	1200	–	–	2	1	14 (2)	3.7 (0.3)	0.31 (0.03)	12.0 (0.1)	5.9 (0.3)	174.6 (35.7)
12	2	2500	Organic	Organic	4	1	14	2.8 (0.2)	0.24 (0.01)	11.6 (0.3)	6.1 (0.1)	120.4 (7.4)
13	3	1500	Organic	–	0	2	20 (2)	2.1 (0.1)	0.22 (0.01)	9.8 (0.0)	5.5 (0.2)	117.8 (17.1)
14	2	700	Organic	–	5	1	30	3.6 (0.5)	0.35 (0.03)	10.4 (0.5)	5.0 (0.2)	205.6 (16.8)

0.45-μm filter in a Büchner funnel. The degreased filter cake was vacuum-dried at 100 mbar in a vacuum oven (Hereaus, Hanau, Germany) at room temperature for 1 h.

2.4.3. Extraction and analysis of total polyphenols

For analysis of the total phenolic compounds, 0.1 g of the homogenized defatted cocoa powder was mixed with 25 mL acetone/H_2O (60/40). Samples were extracted three times by stirring for 15 min on ice, ultrasonic treatment (Sonorex, Super RK 510H, Bandelin, Berlin, Germany), and centrifugation (Thermo Scientific, Mega-Fuge 11 R Centrifuge, Heraeus, Hanau, Germany) at 4100 rpm for 10 min. 2 mL concentrated acetic acid was added to the three combined supernatants. Acetone was removed in a rotary evaporator (LABO Rota SE 320, Resona Technics, Gossau, Switzerland; 40 °C, 60 mbar). The aqueous residue was dissolved in 100 mL of deionized water and frozen.

The total content of polyphenols was analyzed with the Folin–Ciocalteu procedure (Singleton and Rossi, 1965). Measurement of total polyphenols was carried out mixing 1 mL of sample with 0.5 mL Folin–Ciocalteu reagent, adding 2 mL Na_2CO_3 solution (20%) and subsequently 7.5 mL deionized H_2O. The resulting blue complex was stabilized for 10 min at 70 °C. After cooling to room temperature, the absorbance was determined photometrically at λ = 730 nm and 20 °C (Uvikon 943, Double Beam UV/vis Spektrophotometer, Kontron Instruments, Rossdorf, Germany).

2.4.4. Extraction of polyphenolic compounds for chromatographic analysis

For measurement of polyphenolic compounds by RP-HPLC, 0.1 g of fat-free cocoa powder was stirred with 5 mL methanol for about 30 s with an ULTRA-TURRAX T25 agitator (Janke & Kunkel, Staufen, Germany) and afterwards rinsed with 2 mL methanol. The extract was transferred to an ultrasonic bath, cooled for 15 min on ice and centrifuged at 4100 rpm for 10 min. The extraction was repeated twice and the supernatants collected. Methanol was removed from the united extracts in a rotary evaporator (40 °C, 100 mbar) and the residue resolved in 3 mL methanol. Samples were filtered through a syringe filter of 0.45 μm. The concentration of the phenolic compounds was determined by RP-HPLC according to Elwers et al. (2009) and Niemenak et al. (2006). Phenolic compounds were detected at wave lengths ranging from 225 to 540 nm against calibration series of the respective polyphenols at λ = 280 nm.

2.4.5. Analysis of methylxanthines

Methylxanthines (theobromine and caffeine) were extracted from 0.1 g cocoa powder by adding 40 mL boiling deionized water and keeping the samples for 30 min at 100 °C. After cooling the samples to 20 °C, 0.2 mL Carrez I solution (150 g L^{-1} $K_4[Fe(CN)_6]\cdot 3H_2O$) was added followed by clarifying with 0.2 mL of Carrez II solution (300 g L^{-1} $ZnSO_4\cdot 7H_2O$). The samples were diluted to a final volume of 50 mL with deionized water and filtered (0.45-μm syringe filter). Methylxanthines were quantified using RP-HPLC at a wave length of 274 nm against calibration series of theobromine and caffeine.

2.4.6. Analysis of soil parameters

Soil was dried at 105 °C for 24 h and sieved with a motorized sieve at a mesh size of 2 mm.

For the determination of soil pH and electrical conductivity, 10 g of dried and sieved soil was stirred for 1 h in 25 mL of deionized water and of 0.01 mol/L $CaCl_2$ solution, respectively. The pH was measured in both solutions, and electrical conductivity in the water samples only. All analyses were conducted using a VWR sympHony SP90M5 (Radnor, PA, USA) device.

The contents of organic carbon and nitrogen were determined in a ground soil sample (2 g for 2 min in a disk swing mill, Siebtechnik, Mülheim an der Ruhr, Germany). Aliquots of 0.5–0.8 g were transferred to special tin saggers and closed by cold welding prior to analysis. Measurements were conducted in a CN-Analyzer (vario EL cube, Elementar Analysensysteme, Hanau, Germany).

2.5. Statistics

All cover classes from vegetation relevés of the herb and shrub layer were transformed to mean cover percentage following Frey and Lösch (2010) with: *r* = 0.1%; + = 0.5%; 1 = 2.5%; 2 = 15%; 3 = 37.5%; 4 = 62.5%; 5 = 87.5%. These data were used for analyses of species composition and diversity indices. All statistical tests were carried out with R, version 3.1.1 (R Development Core Team, 2014)

Species composition both in the herb and shrub layer and in the shade tree layer as well as influential environmental predictors were visualized using non-metric multidimensional scaling (NMDS) within the R packages *vegan* (Oksanen et al., 2008) and *mass* (Ripley, 2015). This ordination technique offers a pronounced robustness and a high reliability compared to other multivariate methods (Leyer and Wesche, 2007). It is characterized by an iterative approach which ordinates samples (here plots) in a k-dimensional space according to their ranked distances (Leyer and Wesche, 2007). Ordination was carried out based on the Bray–Curtis dissimilarity matrix, which is particularly suitable for ordering sites along gradients due to the beneficial rank order relation (Faith et al., 1987). Consequently, Bray–Curtis dissimilarity is frequently used in publications on community ecology (e.g. Buchholz, 2010). The quality of the ordination was assessed using the stress value (Leyer and Wesche, 2007), which was calculated with *metaMDS*. Ecologically meaningful interpretation of the results is possible at a stress value <0.15. The best solution with minimum stress is selected iteratively based on Procrustes rotations. Calculations were conducted with a maximum of 20 random starts and five dimensions which showed the lowest stress value. To illustrate the relation of species composition with soil and management parameters but also with pathogen incidence and plant secondary compounds in cacao seeds, we fitted environmental data onto the ordination using the function *vector fitting* which performs a Monte-Carlo randomization test (1,000 free permutations of the data) in the package *vegan*. This approach is based on the comparison of the R^2 values of the present dataset to those of the randomized data (Manly, 1997). We included only variables with a *p*-value <0.05.

Alpha diversity was measured in terms of species richness, Shannon ($H = -\sum_{i=1}^{S} p_i \ln p_i$ with p_i: the proportion of species *i* and *S*: the number of species) and Simpson ($D = 1 - \sum_{i=1}^{S} p_i^2$ with p_i: the proportion of species *i* and *S*: the number of species) indices, and evenness (based on the formula: exp(H)/S with H: Shannon entropy and *S*: species richness). Values were calculated separately for the shrub and herb layer and for the shade tree layer in the package *BiodiversityR* (Kindt and Coe, 2005). For the shade trees, abundance-related analyses were calculated based on the basal area derived from the measurements of circumference at breast height.

The entire set of metric variables was analyzed in terms of normality using histograms (Zuur et al., 2009). Values for Simpson index and evenness were arcsin-transformed prior to analyses to normalize data as these indices are scaled as proportion data. We used generalized linear mixed models (GLMM) for selection of variables that influence biodiversity, yield, pathogen incidence, and plant secondary compounds due to the fact that the data structure shows nesting and hence spatial autocorrelation (Zuur et al., 2009) as in some cases more than one plot pair on each farm

and always two plots (CCN-51 and local variety) per pair were analyzed. As an extension of generalized linear models, GLMMs allow for the analysis of data sets that show high levels of heterogeneity and spatial correlation. Since sample units as fixed effects produce various levels in the models which makes interpretation difficult, GLMMs integrate these sampling units and their nested structure as random effects (Zuur et al., 2009). In our models, environmental, management, and biochemical variables along with the genetic background of the cacao trees were included as fixed effects, whereas *farm, plot pair* and *plot* were random effects with *plot* (CCN-51 vs. local variety) nested in *plot pair* and *plot pair* nested within *farm*. Yield, fruit counts, and richness showed Poisson or pronouncedly skewed distribution and were therefore modeled using the *glmer* function in the *lme4* package (Bates, 2010). All other variables were modeled with the *lme* function in the *nlme* package (Pinheiro and Bates, 1995). Interactions among variables were not considered to reduce the potential complexity of the models.

In a first step, we aimed at determining a general prediction model for each variable based on stepwise variable reduction of the entire variable set. In a second step, biodiversity, the genetic background of the cacao trees, plant secondary compounds, pathogen incidence, farm management, and soil parameters were included separately as variable blocks into the models and variables then reduced. Model selection was finally based on minimizing the AIC values.

3. Results

3.1. Impact of biodiversity on yield

Overall, the flora of weeds and coexisting plants in Peruvian cacao farms comprised 112 vascular plant species of which 42 were determined merely to the genus level and 18 species were included as morphospecies into the analyses. Species number per plot ranged from 2 to 18 species. NMDS of species composition of the herb and shrub layer (Fig. 1a) showed direct and indirect correlations with various variables. Longer arrows indicate a higher explained variance and more distanced plots a higher dissimilarity in species composition. Soil conditions such as pH, electrical conductivity, and CN ratio significantly influenced the species composition of the herb and shrub layer. In parallel, a lower soil pH resulted in a higher polyphenol content of the beans. A lower electric conductivity led to higher *Phytophthora* spp. infestation, which is presumably the reason why more pesticides were used. Also further agricultural management parameters (shape pruning, shading of cacao trees) as well as the number of unripe pods per cacao tree were significantly correlated with the species composition.

Alpha diversity of the herb and shrub layer in cacao farms responded to divergent factors. Species richness increased significantly with higher cover of herb layer ($p = 0.0299$), higher insolation of herb layer ($p = 0.0273$), higher farm age ($p = 0.0215$), and the use of chemical pesticides ($p = 0.00319$ for chemical pesticides vs. no pesticides, $p = 0.0059$ for chemical and organic pesticides vs. no pesticides, AIC = 229.0). In contrast, values of Shannon ($p = 0.0014$, AIC = 58.2) and Simpson ($p < 0.0001$, AIC = −8.9) indices and evenness ($p < 0.0001$, AIC = −2.8) were consistently lower at a higher cover of the herb layer.

NMDS of shade tree composition (Fig. 1b) also showed an interaction with management parameters (pruning regime, use of fertilizers), as well as with cacao fruit size and biochemical traits (fruit length and circumference, catechin content). A higher fertilizer use resulted in a lower catechin content in the cacao seeds and is negatively correlated with shape and sanitary pruning. As alpha diversity of shade trees is directly managed by the farmers, we did not test possible drivers of the shade tree diversity.

In general, our results show that biodiversity did not result in a reduction of cocoa yield. We found no evidence that yield per hectare or fruit set (total number of fruits per tree) was negatively affected by higher levels of biodiversity in the herb and shrub layer and in the shade tree layer. Both measures of crop quantity showed differences in terms of fertilizer regime. Yield per hectare responded negatively to organic fertilizer vs. no fertilizer ($p < 0.0001$, AIC = 555.6). Total number of fruits per tree was higher in cases where chemical plus organic fertilizer was applied ($p = 0.0186$), lower in CCN-51 trees compared to local varieties ($p = 0.0129$, Table 2), lower when *M. roreri* incidence decreased

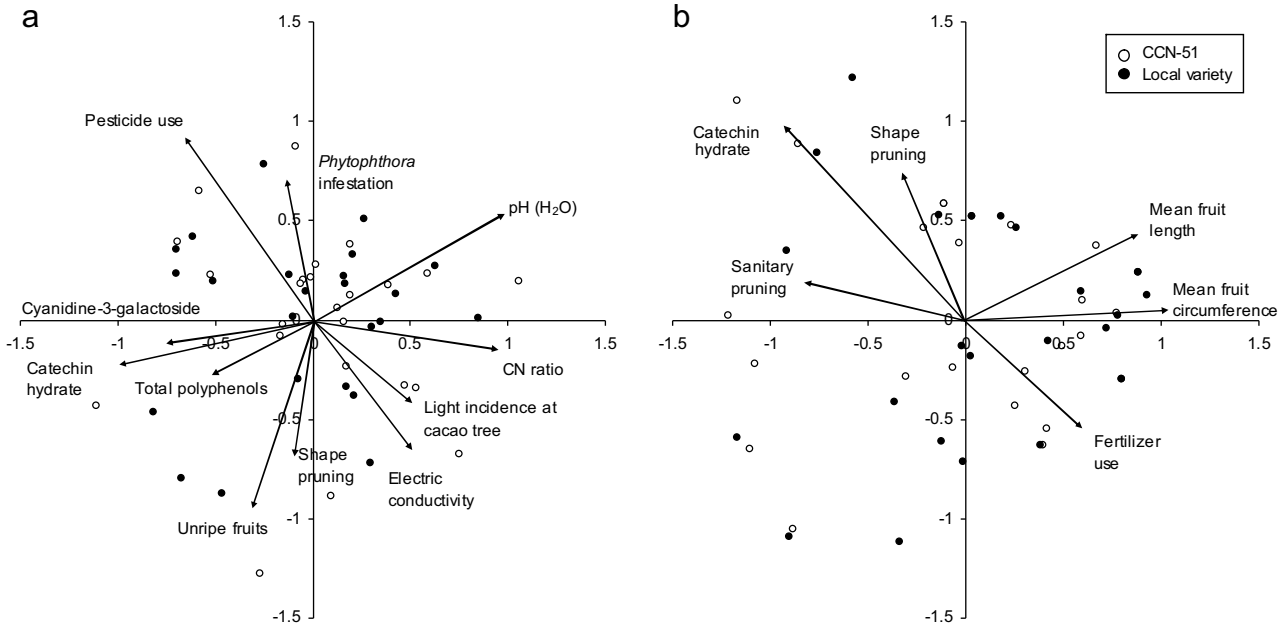

Fig. 1. Correlations of species composition in **a** herb and shrub layer and **b** shade tree layer with soil, management, pathogen incidence, crop yield, and crop quality parameters according to NMDS. Only significant correlations ($p < 0.05$) are displayed. Stress values: a: 0.11; b: 0.10.

Table 2
Fruit number and size, pathogen incidence, plant secondary compounds in cacao seeds, and plant abundance and diversity indices of 48 study trees and plots (means and standard errors of 24 trees/plots of the clone CCN-51 and 24 local varieties). Values in bold with asterisks refer to significant differences between CCN-51 and local varieties in generalized linear mixed models. ffdm = fat-free dry matter.

	CCN-51	Local varieties
Mean number and size of cacao pods per tree		
Total pod number per tree	**16.3 (1.6)**	**23.6 (3.1)***
Ripe healthy pods per tree	4.5 (0.4)	5.8 (1.2)
Unripe pods per tree	**9.8 (1.4)**	**14.1 (1.8)***
Infested pods per tree	1.9 (0.4)	3.6 (1.8)
Mean circumference of ripe pods (cm)	31.0 (0.6)	30.0 (0.7)
Mean longitudinal length of ripe pods (cm)	22.7 (0.5)	21.5 (0.6)
Mean pathogen incidence per tree		
Percentage of infested pods (%)	10.2 (1.8)	11.8 (2.1)
Percentage of pods with *Phytophthora* spp. incidence (%)	8.1 (1.6)	6.5 (1.5)
Percentage of pods with *M. roreri* incidence (%)	2.2 (0.9)	5.3 (2.1)
Number of branches deformed by *M. perniciosa*	1.5 (0.7)	2.3 (0.6)
Plant secondary compounds (means of at least two pods per tree)		
Theobromine (mg/g ffdm)	**26.07 (0.39)**	**28.12 (0.63)***
Caffeine (mg/g ffdm)	5.63 (0.35)	6.80 (0.56)
Ratio theobromine/caffeine	5.0 (0.3)	4.9 (0.5)
Total polyphenols (mg/g ffdm)	49.0 (1.1)	47.1 (1.5)
Catechin hydrate (mg/g ffdm)	1.06 (0.11)	0.87 (0.06)
Epicatechin (mg/g ffdm)	25.93 (1.09)	23.92 (1.78)
Cyanidine-3-galactosid (mg/g ffdm)	0.41 (0.04)	0.45 (0.06)
Cyanidine-3-arabinosid (mg/g ffdm)	0.76 (0.6)	0.96 (0.13)
Mean plant abundance and diversity per study plot		
Basal area of shade trees (m^2/ha)	5.06 (0.94)	5.32 (0.85)
Richness of shade trees per 400 m^2	3.0 (0.5)	3.0 (0.4)
Shannon index of shade trees per 400 m^2	0.58 (0.11)	0.62 (0.10)
Simpson index of shade trees per 400 m^2	0.356 (0.061)	0.399 (0.059)
Mean cover of herb layer (%)	15.5 (3.6)	13.4 (3.4)
Richness of herb and shrub layer per 25 m^2	7.3 (0.5)	7.5 (0.5)
Shannon index of herb and shrub layer per 25 m^2	1.32 (0.10)	1.42 (0.08)
Simpson index of herb and shrub layer per 25 m^2	0.619 (0.045)	0.671 (0.031)
Evenness of herb and shrub layer per 25 m^2	0.588 (0.034)	0.618 (0.039)

($p = 0.0192$), and lower when basal area of shade trees was higher ($p = 0.0231$, AIC = 350.6).

The number of ripe fruits per tree without signs of fungal diseases as well as mean fruit circumference and length responded positively to alpha diversity measures, irrespective of the genetic background of cacao trees (Table 2). Ripe fruits were more abundant as the Shannon and Simpson indices of the herb and shrub layer increased (Shannon index: $p < 0.0001$, AIC = 236.9; Simpson index: $p < 0.0001$, AIC = 236.9). The number of fungus-free ripe fruits was in addition significantly positively related to evenness when combined with herb cover (the latter showing a marginally significant negative correlation) (Fig. 2a). Mean circumference ($p = 0.0143$, AIC = 244.6) and length ($p = 0.0065$, AIC = 232.3) of ripe fruits were significantly larger when the understory vegetation showed a higher evenness. In contrast, increased richness in the shade tree layer led to reduced fruit circumference ($p = 0.0061$, AIC = 248.5) and length ($p = 0.0205$, AIC = 239.6) and to a lower number of unripe fruits ($p = 0.0484$, AIC = 327.9). Similarly, higher basal area of shade trees was accompanied by lower fruit circumference ($p = 0.0271$, AIC = 263.8) and length ($p = 0.0337$, AIC = 253.4) and number of unripe fruits ($p = 0.0012$). The number of unripe fruits was also negatively impacted by organic pesticide use ($p = 0.0117$), and positively impacted by the combined use of chemical and organic fertilizer ($p = 0.0043$) and by cover in the herb layer ($p = 0.0461$; AIC = 325.1). The number of unripe fruits was significantly more abundant in local cacao varieties than in CCN-51 (Table 2). Furthermore, the Shannon index of shade trees was negatively related to mean fruit circumference ($p = 0.0443$, AIC = 249.0). Overall, alpha diversity in the herb and shrub layer proved to have a rather positive impact on the number and size of healthy fruits, whereas abundance and biodiversity in the shade tree layer were negatively related to fruit number and size.

3.2. Impact of biodiversity on pathogen incidence

The overall ratio of infested to healthy pods per tree was related neither to biodiversity nor to specific plant secondary compounds. Still, the positive albeit not significant estimates of the correlation of pathogen incidence with shade tree richness and Shannon index imply that shade tree diversity might result in increased susceptibility to pathogen outbreak. CCN-51 trees showed no difference in infestation rate compared to local varieties (Table 2). Fertilizer use [organic ($p = 0.0306$) and organic with chemical ($p = 0.0110$)] reduced infestation with fungi, whereas organic pesticide use increased infestation rate ($p = 0.0068$, AIC = 337.2).

The separate consideration of each pathogen revealed a more comprehensive view of the complex interactions of cacao trees with coexisting plants. *Phytophthora* spp. infestation was higher when organic pesticides were used ($p = 0.0375$) and lower when caffeine contents were higher ($p = 0.0071$, AIC = 310.0), and when evenness and cover of the herb layer were higher (Fig. 2b). Soil parameters and genetic background did not affect *Phytophthora* infestation (Table 2).

The infestation rate with *M. roreri* was more pronounced at high evenness of the herb and shrub layer (Fig. 3a). Cacao trees with higher epicatechin content showed lower levels of this fungus (Fig. 3c). However, epicatechin content was not significantly correlated with evenness of the herb and shrub layer (Fig. 3b). In addition, fertilizer use (irrespective of type) significantly decreased

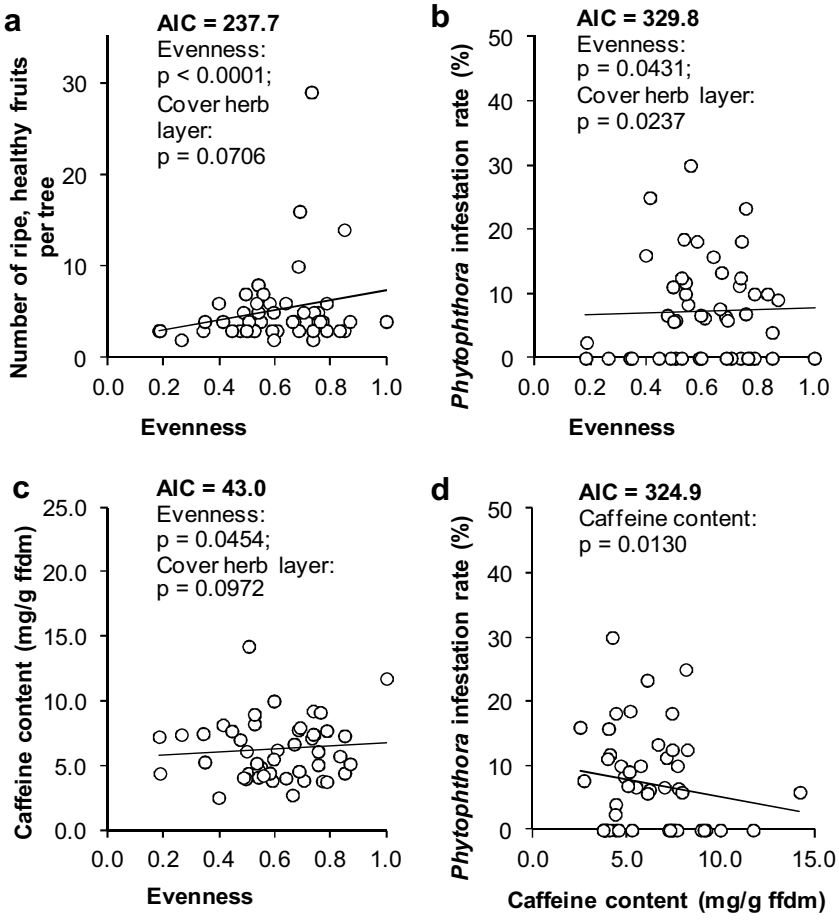

Fig. 2. Correlations of **a** the number of ripe fruits per tree (glmer (Fruit number ~ Evenness + Cover herb layer + (1|Farm/Plot pair/Plot), family = "poisson"), **b** *Phytophthora* incidence (lme (*Phytophthora* ~Evenness + Cover herb layer, random = ~1|Farm/Plot pair/Plot), and **c** caffeine content with evenness of herb and shrub layer (lme (Caffeine content ~Evenness + Cover herb layer, random = ~1|Farm/Plot pair/Plot); **d** correlation of caffeine content with *Phytophthora* incidence (lme (*Phytophthora* ~Caffeine content, random = ~1|Farm/Plot pair/Plot). Model documentation according to GLMM. ffdm = fat-free dry matter. Lines show linear regression of the respective variables to indicate the slope of significant correlations.

M. roreri incidence (AIC = 328.7). Again, CCN-51 did not differ from the local varieties in terms of *M. roreri* infestation (Table 2).

Local varieties showed a marginally higher rate of infestation with *M. perniciosa* in comparison to CCN-51 (Table 2). Both total phenolic compounds and cyanidine-3-glycosides were higher at higher levels of infestation with witches' broom (Fig. 4c). A higher electrical conductivity in soil reduced infestation rate ($p = 0.0212$, AIC = 238.3). In contrast to the other two pests, biodiversity indices were not related to *M. perniciosa* incidence. Less light at the herb layer ($p = 0.0013$), more light at the shrub layer ($p = 0.0095$), or higher cyanidine-3-glycoside content ($p = 0.0246$) were related to a higher infestation rate (AIC = 246.2), which points to certain

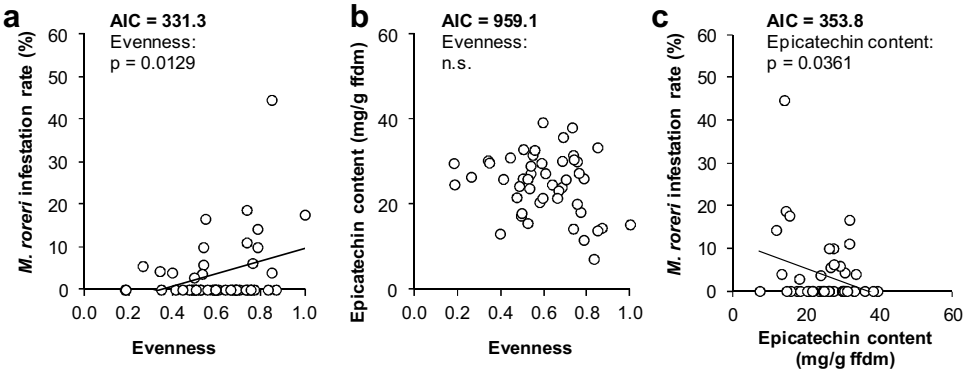

Fig. 3. Correlations of **a** *Moniliophthora roreri* incidence (lme (*Moniliophthora* ~Evenness, random = ~1|Farm/Plot pair/Plot) and **b** epicatechin content with evenness of herb and shrub layer (lme (Epicatechin ~Evenness, random = ~1|Farm/Plot pair/Plot); **c** correlation of epicatechin content with *M. roreri* incidence (lme (*Moniliophthora* ~Epicatechin content, random = ~1|Farm/Plot pair/Plot). Model documentation according to GLMM. ffdm = fat free dry matter. Lines show linear regression of the respective variables to indicate the slope of significant correlations.

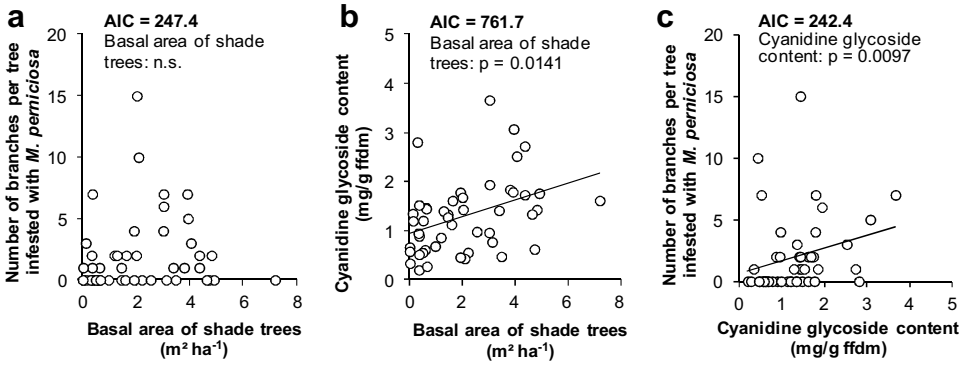

Fig. 4. Correlations of **a** *Moniliophthora perniciosa* incidence (lme (*M. perniciosa* ~Basal area of shade trees, random = ~1|Farm/Plot pair/Plot) and **b** cyanidine glycoside content with basal area of shade trees (lme (Cyanidine glycoside ~Basal area of shade trees, random = ~1|Farm/Plot pair/Plot); **c** correlation of cyanidine glycoside content with *M. perniciosa* incidence (lme (*M. perniciosa* ~Cyanidine glycoside, random = ~1|Farm/Plot pair/Plot). Model documentation according to GLMM. ffdm = fat free dry matter. Lines show linear regression of the respective variables to indicate the slope of significant correlations.

impacts of the tree and herb cover on pathogen incidence. However, these findings were not consistent when cover and basal area data were tested directly (see Fig. 4a).

Overall, the different pathogens were controlled by divergent factors related to soil and farm management. Plant diversity in the herb and shrub layer of cacao fields reduced pathogen incidence of *Phytophthora* spp., whereas *M. roreri* was even more frequent in farms with higher herb and shrub evenness.

3.3. Impact of biodiversity on plant secondary compounds

The total content of polyphenols in cacao seeds did not respond to biodiversity or to biomass of either vegetation layer. Catechin hydrate alone, however, showed a significant positive correlation with species richness in the herb and shrub layer ($p = 0.0354$; AIC = 707.8), whereas epicatechin ($p = 0.0087$; when modeled together with shape pruning, $p = 0.0145$, sanitary pruning, $p = 0.0048$, electric conductivity, $p = 0.0009$, and *M. roreri* incidence, $p = 0.0132$, AIC = 915.2) and cyanidine glycosides increased with the basal area of shade trees (Fig. 4b). Surprisingly, there was no significant difference in polyphenol content between local cacao varieties and CCN-51. Instead, most polyphenols were related to pathogen incidence and soil conditions.

Total content of polyphenols was significantly correlated to soil pH (negative correlation, $p = 0.0105$) and *M. perniciosa* occurrence (positive correlation, $p = 0.0148$, AIC = 309.5). In contrast, epicatechin was related to the presence of *M. roreri* and electrical conductivity (see above), whereas catechin hydrate was higher on farms with shape pruning ($p = 0.0175$, combined with a marginally significant effect of sanitary pruning, AIC = 697.3). Cyanidine glycosides decreased with electrical conductivity in soil ($p = 0.0084$) and increased in fields treated with chemical pesticides ($p = 0.0229$) and higher basal area of shade trees ($p = 0.0013$, AIC 710.3). In addition, *M. perniciosa* was associated with increased concentrations of cyanidine glycosides in cacao seeds ($p = 0.0415$, AIC 751.6).

There was no significant relationship of biodiversity indices with the total content of methylxanthines. Still, the content of methylxanthines was significantly different between the high-yield clone CCN-51 and local cacao varieties ($p < 0.0001$, AIC = 48.8) and was higher in the presence of *M. perniciosa* ($p = 0.014$, AIC = 58.0). Theobromine and caffeine showed clear differences in their reaction to the parameters considered. Theobromine content responded positively to richness in the herb and shrub layer ($p = 0.0385$), negatively to the abundance of shade trees ($p = 0.0126$), and was higher in fields with regular sanitary pruning ($p = 0.0335$) and in local varieties ($p = 0.0043$, AIC = 34.6). Accordingly, the Shannon index in the herb and shrub layer also had a marginally significant positive correlation to theobromine. Pathogen incidence was not related to the content of theobromine, but a higher soil pH led to lower theobromine contents ($p = 0.0178$, AIC 34.9).

Caffeine content increased with higher evenness and cover of the herb layer (Fig. 2c). In addition, caffeine content was higher after application of organic pesticides ($p = 0.0089$) and at more pronounced *M. perniciosa* incidence ($p = 0.0411$) and lower after application of mixed fertilizers ($p = 0.0388$), whereas trees characterized by a higher content in caffeine were less affected by *Phytophthora* spp. ($p = 0.0095$, AIC = 35.2).

4. Discussion

Our data elucidate the complex interplay of yield, pathogen incidence and plant secondary compounds with plant diversity in tropical agroforestry systems. In contrast to the results of Clough et al. (2011), we found no negative impact of herb diversity on yield but a positive relation of herb and shrub alpha diversity measures on pod size and the number of healthy fruits as also described by Deheuvels et al. (2012). Abundance and diversity of shade trees showed a negative correlation to the size and number of ripe fruits as well as to fruit set, which has been similarly shown in other studies (Gidoin et al., 2014; Koko et al., 2013). However in accordance with Clough et al. (2011) and Somarriba and Beer (2011), there was no correlation of tree species and overall yield. The inclusion of different diversity measures that take species evenness into account and of biomass indicators in each vegetation stratum revealed important insights into the mechanisms of biodiversity effects in agroecosystems. In particular, the role of pathogen incidence and accumulation of plant secondary compounds in cacao seeds in biodiversity-yield relationships can be assessed based on our data, which is a novel aspect in biodiversity studies (Samedani et al., 2014).

4.1. Impact of biodiversity on yield

Species number in the herb and shrub layer of cacao farms was intermediate compared to other studies (Cicuzza et al., 2011, reported 91 herb species on 43 cacao farms in Sulawesi; Bisseleua and Vidal, 2008, found 260 herb species on 17 farms in Cameroon). In accordance with Cicuzza et al. (2011), herb and shrub richness was most clearly related to light incidence, but also to the cover of the herb layer and field age. Surprisingly, there was a positive

correlation with chemical pesticides, which implies that intense management increases species number—presumably due to canopy opening in the course of pruning and shade tree reduction.

We found clear evidence for positive impacts of plant diversity in the herb and shrub layer on the productivity of cacao trees (number and size of healthy pods) in the study area. Such positive relationships among herb species and crop yield in tropical perennial production systems have also been demonstrated in oil palm fields by Samedani et al. (2014) and in banana and coconut fields by Cierjacks et al. (2016). These biodiversity effects may be a consequence of different plant functional types in the tree-dominated crop layer compared to the herb and shrub layer, which enhance complementarity among plants in terms of resource acquisition (e.g., Karanika et al., 2007; Smith et al., 2010). Complementarity is expected to be more pronounced under resource limitation than under good nutrient supply (see Brooker et al., 2008; Mulder et al., 2001) and after a prolonged interaction time (Cardinale et al., 2007), as also seems to be the case in our study with highly nutrient-limited soils and a perennial crop species. However, there was no correlation of field-wide yield data and total fruit set per cacao tree with herb-layer biodiversity. This is in contrast to Clough et al. (2011), presumably owing to the rather coarse information on yield given by the farmers in our study and the inclusion in the fruit set data of unripe fruits, some of which will perish from fungal infestations and hence not contribute to cocoa yield. While chemical fertilizer use significantly increased fruit set (see also Uribe et al., 2001), the application of organic fertilizer reduced yield, in contradiction to the results of other studies (Krauss and Soberanis, 2002). The use of pesticides, with their inherent hazards to humans and the environment (Tijani, 2006), was not beneficial to yield parameters in our study area and may therefore be ceased.

Biomass of the herb and shrub layer had a marginally negative impact on the number of ripe fruits. This result shows that an herb and shrub layer composed of only a few dominant species may foster competition rather than complementarity among plants within agroecosystems, which can make the use of herbicides necessary (Cierjacks et al., 2016). Despite the weak correlation in the model, the negative relationship between herb cover and evenness-related biodiversity indices points to pronounced dominance of certain species in plots with high cover values. As evenness-related biodiversity indices and cover showed contrasting effects in our models (see Fig. 2a), the observed biodiversity effect seems rather related to complementarity based on the interaction of different evenly distributed species as opposed to a selection effect which is caused by few species with particular traits (see Cardinale et al., 2007). However, our study design does not allow for the differentiation of complementarity and selection effects (Loreau and Hector, 2001).

Beyond simple diversity, the species composition in the herb and shrub layer also showed correlations with the number of unripe fruits and with polyphenols and *Phytophthora* incidence. Hence, there is some evidence that the plant assemblage influences the outbreak of fungal diseases. An interaction of plant secondary compounds, such as polyphenols and methylxanthines, and pathogens was shown for all pathogen species considered, although the correlation analysis failed to prove a direct relation between *Phytophthora* and polyphenols as suggested by the NMDS (see Fig. 1a). The NMDS results may therefore be explained by indirect effects of soil conditions and management on the species assemblage and pathogen incidence and on crop yield and quality parameters. For instance, the relation of fruit set (number of unripe fruit) and species composition may be a consequence of management measures such as pruning which could have caused higher fruit set and shifts in the species' occurrence and abundance. Still, the NMDS implies a tight interplay of herb species, pathogen incidence and cocoa production. Consequently, the biodiversity effects in our case may be related to indirect interactions via pathogens in addition to possible direct complementary plant-plant interactions. However, the models on yield parameters which included pathogen incidence showed high AIC values and mostly no correlations between them (data not shown). Therefore, pathogen outbreaks seem to be efficiently controlled in the studied farms, which prevents pronounced losses in fruit set and yield. This is supported by the low percentage of pods per tree infested with *Phytophthora* spp. or *M. roreri* (compared to Krauss and Soberanis, 2002; Soberanis et al., 1999) and the nearly entire absence of pods infested with *M. perniciosa*.

The overall negative impact of shade trees on fruit size, fruit set, and the number of unripe fruits adds evidence to the controversial role of shading in cacao farms. In accordance with our data, light is most commonly regarded as positive for cacao production—particularly when there is no limitation in nutrients (Beer et al., 1998; Clough et al., 2011; Daghela Bisseleua et al., 2013; Koko et al., 2013; Wade et al., 2010). Still, cacao trees often show reduced vitality in full-sun fields and must be replaced regularly in such cultivation schemes (Beer et al., 1998). Moreover, beneficial insects such as spiders and wasps are known to be supported by shade trees (Daghela Bisseleua et al., 2013; Stenchly et al., 2011), and the negative effect of shading on cocoa yield can be ameliorated by allowing adequate spacing (Koko et al., 2013) and choosing species with a moderate canopy cover (Gidoin et al., 2014; Ratnadass et al., 2012; Somarriba et al., 2013). These factors indicate that it is possible to achieve diversity in the shade tree layer without significantly compromising yield (as proposed by Somarriba and Beer, 2011; Tscharntke et al., 2011). Interestingly, both shade tree abundance and diversity proved to negatively influence fruit size and fruit set in our study, which is in line with Gidoin et al. (2014) and Wade et al. (2010) and may be attributed to the fact that a more diverse tree layer is also denser. The NMDS results of the tree layer (see Fig. 1b) show that the composition of the shade tree layer is related to fruit size and to plant secondary compounds, whereas the observed correlation with the management parameters (pruning and fertilizer use) implies that intense management is reflected in a certain shade tree composition. Overall, shade trees seem to exhibit a rather negative effect on cocoa yield, which is in accordance with other studies. In contrast, a species-rich herb and shrub layer fosters the development of healthy pods in the study region.

4.2. Impact of biodiversity on pathogen incidence

Our results clearly highlight the divergent behavior of the pathogen species considered, which makes it difficult to find general drivers of fungal infestation. As found by Beer et al. (1998), shade trees together with higher humidity increased the incidence of fungal pathogens, although the correlations in our study were only marginally significant. Consequently, shade management seems relevant for preventing fungal diseases in cacao as frequently mentioned by other authors (e.g. Acebo-Guerrero et al., 2012), but a direct correlation between pathogen incidence and shading is often difficult to find (see also Daghela Bisseleua et al., 2013). Gidoin et al. (2014) found a negative correlation of pest incidence and shade tree density, but the spatial structure of the shade trees had a much greater impact than density alone, which again provides evidence for the complex interplay of pathogen incidence and shade trees.

As already stated by Krauss and Soberanis (2002), the use of fertilizers significantly reduced overall pathogen incidence and in particular that of *M. roreri*, which may be explained by the greater vitality of fertilized trees and improved N supply for the production of plant secondary compounds that serve as a defense against

fungi. The decrease in *M. perniciosa* at higher soil pH points to a similarly beneficial effect of the nutrition status and resistance of cacao trees. In contrast, the pesticides used did not lead to a decrease in pathogen incidence, and other management measures seem more successful for disease regulation (see Soberanis et al., 1999).

Biodiversity in the herb and shrub layer was correlated with a lower incidence of *Phytophthora* but a higher incidence of *M. roreri*. Hence, biodiversity affected different pathogens in a different way and pathogen species which are competing for the same resources may also be interrelated. In addition, the pathogens responded differently to plant secondary compounds with *Phytophthora* being less frequent on trees with high caffeine content and *M. roreri* less frequent on trees with high epicatechin content. In contrast, *M. perniciosa* incidence was associated with higher production of total polyphenols and in particular of cyanidine glycosides.

These results show that biodiversity does not necessarily counteract the development of all pest and disease species as proposed by e.g. Palm et al. (2014) but rather leads to shifts in the pathogen community (see Gosme et al., 2012; Ratnadass et al., 2012). In our case, the more common *Phytophthora* was down-regulated in favor of the less common *M. roreri*, which led to a more evenly distributed pathogen assemblage with a lower risk of mass outbreaks. As expected in the optimal defense theory (Zangerl and Rutledge, 1996), defense against the pathogens with a high infestation risk (*Phytophthora*, *M. roreri*) is presumably constitutive as shown by a negative correlation of plant secondary compounds with pathogen incidence; whereas *M. perniciosa*, which was nearly absent in pods and occurred in only 52.1% of the studied trees, showed an induced defense response with a positive correlation of plant secondary compounds and pathogen occurrence. However, the inducibility was not experimentally tested, and induction with jasmonic acid or salicylic acid may reveal further insights into this hypothesis (Moreira et al., 2014).

A higher diversity of plants and the related fauna is generally assumed to counteract mass development of pests as has been shown for instance for insect food webs where the presence of predators and parasitoids inhibits the dominance of single species (Gosme et al., 2012; Daghela Bisseleua et al., 2013; Puech et al., 2014). In addition, there may also be a direct link between insect and fungi diversity: Wielgoss et al. (2014) assumed that certain ant species may promote fungal infestation by spore dispersal in cacao farms, but the presence of such a relationship in Peru was not investigated here.

Overall, this study shows the dependence of pathogen incidence on diversity and plant species composition with a clearly beneficial effect of biodiversity against the development of common *Phytophthora* spp. Consequently, owing to the efficient reduction of dominant pathogen species, diverse agroforestry systems can be expected to exhibit a lower susceptibility to pathogens.

4.3. Impact of biodiversity on plant secondary compounds

The effects of environmental and management conditions on crop yield and pest abundance have been documented by various studies (e.g. Beer et al., 1998; Deheuvels et al., 2012; Sabatier et al., 2013; Schwendenmann et al., 2010), whereas possible effects on product quality and secondary compounds have as yet scarcely been considered (Bartomeus et al., 2014; Kooyers et al., 2014). Interestingly, we found a combination of different plant secondary compounds in cacao, accumulated presumably both as constitutive and induced defenses, a common strategy in many plant species (Poelman et al., 2009; Röder et al., 2011).

There were clear relationships of biodiversity and biomass indicators with different polyphenols in cacao seeds. Catechin increased with richness in the herb and shrub layer, whereas cyandine glycoside and epicatechin contents were higher at a greater basal area of shade trees. The latter acts possibly as a constitutive defense metabolite against *M. roreri*, which suggests a certain effect of shading on the resistance against this pathogen. These divergent responses in individual secondary compounds are the reason why there was no significant response to plant diversity and biomass in total content of phenolic compounds.

However, polyphenols were additionally related to pathogens and soil conditions. Total polyphenols increased in response to *M. perniciosa* infestation and were also more abundant at lower pH values. A low soil pH is known to cause aluminum toxicity and reduced phosphate availability and often indicates nutrient depletion (Horn et al., 2010), which may impose stress on cacao trees and induce polyphenol synthesis. Accordingly, epicatechin and cyanidine contents responded to electrical conductivity apart from the above discussed correlations with pathogen species.

The content of methylxanthines was more clearly related to the genetic background of the cacao plants. Still, total methylxanthine and caffeine contents were also positively correlated with *M. perniciosa* with caffeine presumably providing constitutive protection against *Phytophthora*. Both methylxanthines increased with the diversity of the herb and shrub layer, and theobromine content decreased with the cover of shade trees. Moreover, the content of theobromine was higher at low pH values, but there was no relation to any of the pathogens considered.

These results show that biodiversity modulates the profile of plant secondary compounds. This may be related to direct allelopathic interaction of cacao with other plants (Fernandez et al., 2013; Pierik et al., 2013), but there is as yet little scientific evidence of such. A more probable explanation is an indirect interaction of the secondary metabolism with pathogens that respond to different diversity levels in the flora and the related fauna. Furthermore, soil conditions proved to influence both the species composition in the herb and shrub layer and the amount of secondary compounds. Consequently, the observed correlation of biodiversity and secondary metabolism appears to be a matter of the associated pathogen assemblage and soil conditions. Still, the plasticity of biochemical traits both in the high-yield clone and in local varieties highlights that crop quality may be influenced by the farming conditions, a factor which has not yet been considered for increasing quality in cocoa production.

4.4. The relevance of the genetic background

In contrast to our expectations, there was only weak evidence for differences in terms of pathogen resistance and plant secondary compounds between CCN-51 and local varieties preserved by the farmers in the area. Contents of methylxanthines and cyanidine glycosides were slightly higher and those of other polyphenols lower in local cacao compared to CCN-51, but neither significantly (see Table 2). In terms of phenolic contents our results matched with other studies that equally found no differences between cacao types (Elwers et al., 2009; Niemenak et al., 2006). The content of theobromine was significantly higher in local varieties but no differences in caffeine content or theobromine/caffeine ratio could be found, the latter again opposed to other studies (Brunetto et al., 2007). This may be attributed to the fact that possible differences may have been masked by the overall greater variability in the local varieties, which is supported by a generally higher standard error in this group (see Table 2).

There was a significant difference toward a higher total pod number per tree as well as toward unripe fruits in local cacao trees. Furthermore, we found a not significantly greater infestation with *Moniliophthora* species in local varieties whereas sensitivity to *Phytophthora* spp. seemed less pronounced compared to CCN-51.

Consequently, at least a subset of the local varieties shows a potential for higher yield, enhanced resistance to pathogens and a more favorable profile of plant secondary compounds. The propagation and distribution of particularly well-producing trees seems feasible based on our data.

5. Conclusion

This study adds evidence for strong plant diversity effects in cacao agroforestry systems. It represents one of the few studies on biodiversity effects in cacao conducted in the neotropics, the species' native region and where a high genetic variability is still maintained. In accordance with other studies, we found overall negative consequences of the shade tree layer on the number and size of healthy, ripe pods. However, there were clear positive impacts of the herb and shrub layer on the size and number of ripe fruits and on resistance against *Phytophthora* spp. In addition, a diverse herb and shrub layer proved to increase methylxanthines, whereas the shade tree layer increased polyphenols such as epicatechin. Overall, the influence of environmental factors seemed to be more relevant than the differences between the high-yield CCN-51 and local varieties, which implies a high potential for quality-optimized cocoa production that has not yet been exploited.

Ensuring a sufficient nutrient supply through soil or fertilizer addition was in general beneficial to yield and pathogen resistance. Furthermore, a cautious shade management with an open shade tree layer along with shape and sanitary pruning seems to contribute to resistance against pathogens and yield parameters. Such measures also changed the profile of secondary compounds in seeds to higher contents of methylxanthines and certain polyphenols. Accordingly, these management measures combined with the maintenance of a high herb and shrub diversity proved to positively affect both crop yield and quality and may thus provide farmers with a more stable income, while improving biodiversity conservation in Peruvian cacao agroforests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2016.02.006.

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