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European Journal of Agronomy 149 (2023) 126890

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Cultivation potential of *Vanilla* crop wild relatives in two contrasting land use systems

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ARTICLE INFO

Keywords:
Agroforestry
Conservation
Crop diversification
Plant performance
Sustainable land use
Vanilla crop wild relatives
Vanilla planifolia

ABSTRACT

Vanilla is an important cash crop for many smallholder farmers around the tropics, and a highly appreciated spice used in a wide range of products. The crop species *Vanilla planifolia* is, however, facing a number of threats that are jeopardizing a stable vanilla supply. The neotropical realm holds at least 37 fragrant *Vanilla* species besides *V. planifolia*. These so-called *Vanilla* crop wild relatives (CWRs) possibly possess interesting traits for crop improvement and market diversification, but received meager attention so far. The aim of our study was therefore to provide insights into the cultivation potential of four *Vanilla* CWRs (*Vanilla hartii*, *V. odorata*, *V. pompona* and *V. trigonocarpa*) naturally growing within our study region in southern Costa Rica, by comparing their plant performance and aromatic profiles with *V. planifolia* and a commercially used hybrid, and this in two types of land use systems where vanilla could possibly be introduced. As such, we established six field sites in secondary forests (SF), adding economic value to these often undervalued ecosystems, and six in existing cacao plantations (CP), diversifying monocrop agrosystems. First, we measured plant survival, vitality and growth over a period of two years, and compared these plant performance traits among *Vanilla* species and land use systems using (generalized) additive mixed models. The models also enabled us to observe possible effects of additionally measured, site-specific environmental variables on vanilla plant performance. Second, we evaluated the aromatic potential of *Vanilla* CWR pods using high-performance liquid chromatography. We found that the hybrid had the highest overall plant vitality and growth, and this in both CPs and SFs, while *V. planifolia* was characterized by a rather low vitality, but high growth rates, and clearly performed inferior in SFs. The CWRs had a good overall vitality, and this in the two land use systems, but were characterized by lower growth rates compared to the hybrid and *V. planifolia*. The aromatic profiles of the *Vanilla* CWR pods indicated potential for market diversification, with *V. odorata* highly resembling the profile of the commercially used species. The other CWRs had varied profiles, with especially *V. hartii* containing high anisyl contents interesting for niche markets. Based on our assessment of *Vanilla* CWR plant performance and aromatic profiles, we see potential for their integration within the two studied land use systems. Corresponding planting designs should, however, take into account species-specific light intensity preferences, monitor chemical properties and the presence of growth-promoting and disease-suppressive soil microbiota, and incorporate vanilla tutor and shade trees with optimal functional traits. We developed a possible vanilla cultivation design that could be implemented for further evaluation in terms of production feasibility.

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https://doi.org/10.1016/j.eja.2023.126890
Received 8 December 2022; Received in revised form 31 May 2023; Accepted 5 June 2023
Available online 15 June 2023

1. Introduction

Crop domestication, defined as the selection of crop species with valuable agronomic traits, has played a central role in modern civilization (Gepts, 2004; Chen et al., 2021). This selection process, however, greatly narrowed the genetic base of much of the cultivated germplasm – known as the "domestication bottleneck" (Tanksley and McCouch, 1997; Meyer and Purugganan, 2013) – and resulted in a lower resilience to (a)biotic stressors (Ross-Ibarra et al., 2007; Van Heerwaarden et al., 2011). A reduced genetic diversity has been observed in many modern crop species, and is exacerbated by the demand for high productivity and uniformity (Dempewolf et al., 2017). Besides, most crops were introduced in areas outside their center of origin, but only part of a crop's diversity was brought over, confining the crops' genetic base in these areas of introduction, and causing an additional "dispersal bottleneck" (Zeder et al., 2006). Crop wild relatives (CWRs), i.e. plant species that are genetically related to cultivated crops, retain high levels of genetic diversity, mainly due to their long evolutionary history across diverse environments (Maxted et al., 2006; Redden and Yadav, 2015). Consequently, they may possess traits that prove useful for crop improvement (Maxted et al., 2007; Vincent et al., 2013; Dempewolf et al., 2017). CWRs are commonly defined using the gene pool concept of Harlan and de Wet (1971). The primary gene pool includes wild populations of the species from which the crop was once domesticated. Secondary and tertiary gene pools refer to closely related species, whereby gene transfer to the cultivated germplasm is possible through conventional breeding or more advanced engineering techniques (Meilleur and Hodgkin, 2004; Maxted et al., 2006; Khan et al., 2020). Recently, and mainly due to rising problems within modern food production systems, there is a growing interest towards the use of CWRs in crop improvement and breeding to guarantee food security (Hodgkin and Hajjar, 2007; Yumurtaci, 2015; Goettsch et al., 2021).

Vanilla, for example, is a highly appreciated spice derived from the fruits, better known as pods or beans, of species belonging to the orchid genus *Vanilla*. It is an important cash crop for several smallholder farmers around the tropics, but is facing many threats provoked by (i) genetic erosion of the crop species *Vanilla planifolia* Andrews, and (ii) vulnerable production systems that allow a rapid spread of pests and diseases (Schlüter et al., 2007; Bory et al., 2008; Flanagan and Mosquera-Espinosa, 2016). A number of studies underlined the urgency of developing a vanilla breeding program that encourages the integration of *Vanilla* CWRs (e.g., Flanagan and Mosquera-Espinosa, 2016; Watteyn et al., 2020; Bramel and Frey, 2021; Chambers et al., 2021; Goettsch et al., 2021). Yet, the majority of incentives exploring the use of *Vanilla* CWRs to counteract aforementioned production problems took place outside the center of origin of the vanilla crop (Neotropics), and emphasis has mainly been laid on the primary CWR gene pool (e.g., Koyyappurath et al., 2015; Grisoni and Nany, 2021). The secondary or tertiary gene pools have received meager attention so far. They may, however, possess a broad spectrum of traits useful to overcome agronomic challenges, especially in times of climate change, as well as to diversify the vanilla market (Flanagan and Mosquera-Espinosa, 2016; Bramel and Frey, 2021; Perez Silva et al., 2021).

The Neotropics host at least 37 fragrant *Vanilla* species besides the crop species *Vanilla planifolia* Andrews (Karremans et al., 2020). These secondary *Vanilla* CWRs are naturally growing in a variety of ecosystems, from primary to secondary forests, and from ridges to wetlands (Soto Arenas and Dressler, 2010). As such, each species may require a particular environmental setting (e.g., specific light intensities and soil characteristics, temperature and precipitation seasonality). Furthermore, vanilla orchids are known to depend on certain microbiota for optimal seed germination and growth (Porras-Alfaro and Bayman, 2007; Johnson et al., 2021) – interactions that may be species-specific as well. Traditional cultivation methods generally integrate the vanilla crop in secondary forests or intercropping agroforestry systems (Osterhoudt and Dove, 2017; Hänke et al., 2018; Havkin-Frenkel and Belanger, 2018; Martin et al., 2020, 2022). The latter intents to mimic the environmental conditions vanilla plants depend on, and creates a buffer against (a)biotic stressors as well as market fluctuations (i.e. through the provision of a variety of crops). Nowadays, however, commercial vanilla plantations range from systems using living tutor trees that provide shade and vanilla vine support, to greenhouses using shade cloths and artificial support poles (Havkin-Frenkel and Belanger, 2018; Osewold et al., 2022). These uniform systems undoubtedly lead to higher production (Martin et al., 2021), yet, require more input to mimic the aforesaid interactions between vanilla plants and their environment, and tend to be more susceptible for diseases (e.g., root and stem rot caused by *Fusarium oxysporum*) (Flanagan and Mosquera-Espinosa, 2016).

Considering future threats related to climate change, we emphasize the need for innovation at both crop species and system level, and see potential in the integration of *Vanilla* CWRs within resilient vanilla production systems. Due to the vulnerable or endangered status of most *Vanilla* CWRs on the IUCN red list (Bramel and Fey, 2021; Goettsch et al., 2021), however, a combined approach of conservation and sustainable production will be key, as suggested by Flanagan and Mosquera-Espinosa (2016) and Watteyn et al. (2020), amongst others. Following their suggestions, wild *Vanilla* populations should be protected within their natural habitat, while *Vanilla* CWRs could be cultivated in the surrounding human-modified landscape (Fig. 1). For example, fallow lands could be reforested with the inclusion of vanilla as the main cash crop, monocrop agrosystems could be transformed to diverse agroforestry systems including vanilla, or vanilla could be cultivated in existing secondary forests to add economic value to these often undervalued ecosystems. Yet, to provide practical guidelines for the integration of *Vanilla* CWR within these plausible cultivation areas, we need to assess *Vanilla* CWR's cultivation potential, which comprises an evaluation of their plant performance (i.e. survival, vitality, growth, production) as well as aromatic profiles (i.e. aromatic compounds of interest to the market).

The aim of our study was therefore to provide insights into the cultivation potential of four *Vanilla* CWRs naturally growing within our study region in southern Costa Rica: *Vanilla hartii* Rolfe, *V. odorata* C. Presl., *V. pompona* Schiede and *V. trigonocarpa* Hoehne, by comparing their plant performance and aromatic profiles with the crop species *V. planifolia* and a commercially used hybrid (*V. planifolia* x *V. pompona*) x *V. planifolia*, and this in two of the aforementioned types of land use systems where vanilla could possibly be introduced (cacao plantations and secondary forests). We formulated the following three research questions: (i) Does vanilla plant performance vary among *Vanilla* species (and more specifically between the CWRs and the commercially used species), and is there a difference between the two selected land use systems?, (ii) Besides species and land use system, do site-specific environmental variables (e.g. light intensity, physicochemical soil properties, soil microbiota) affect vanilla plant performance?, and (iii) Are the aromatic profiles of *Vanilla* similar to the ones of the commercially used species – in other words, could they be of interest to the industry?. The results from our study will be compiled into a cultivation design that integrates *Vanilla* CWRs, besides the commercially used species, within the studied land use systems. This design may serve as a stepping stone for further evaluation in terms of production feasibility, more specifically yields, costs and revenues, and eventually its application in other regions across the center of origin of aromatic *Vanilla* species (neotropics).

2. Materials and methods

2.1. Study area and species

Our study took place in the Área de Conservación Osa (ACOSA) (Fig. 2A), a biodiversity hotspot in southern Costa Rica and home to at least seven fragrant *Vanilla* species (Karremans et al., 2020; Watteyn et al., 2020). ACOSA, classified as a conservation priority area

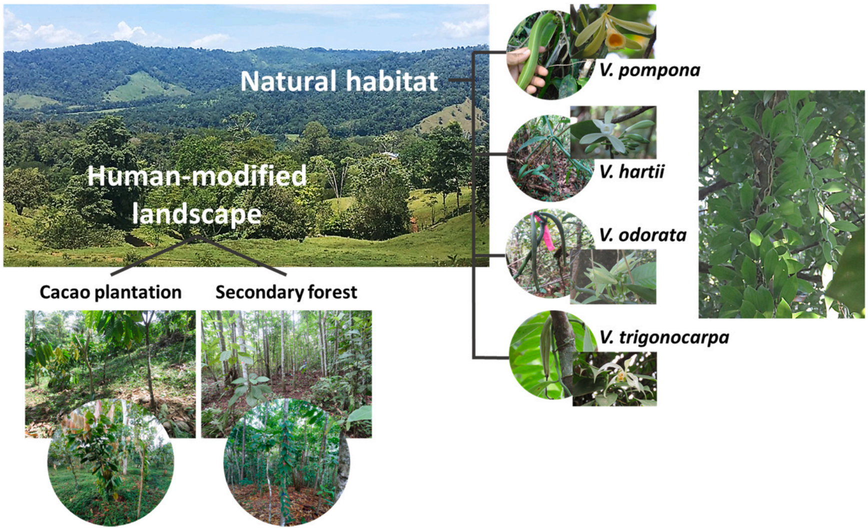

Fig. 1. Conceptualization of the joint approach of *Vanilla* CWR conservation and sustainable cultivation in a landscape of the neotropics (here: southern Costa Rica). Wild populations of *Vanilla* CWRs (i.e. the wild *Vanilla* gene pool) should be protected within their natural habitat, while simultaneously encouraging the integration of promising *Vanilla* CWRs in plausible cultivation areas within the surrounding, human-modified landscape, which could be existing agrosystems (e.g. cacao plantations) or secondary forests (i.e. forests that are naturally regenerating after a significant disturbance), amongst others.

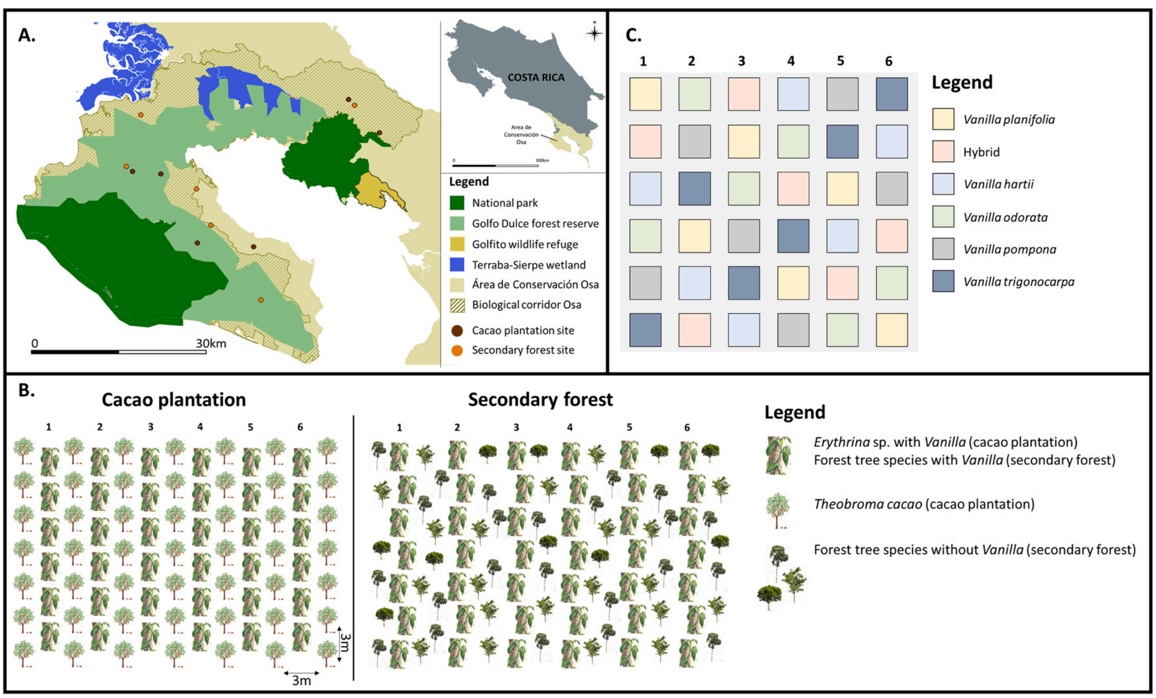

Fig. 2. (A) Location of the 12 field sites across our study area ACOSA. (B) Planting design in traditional 3 m × 3 m cacao plantations (3 m × 3 m refers to the distance between cacao trees in the same and adjacent rows) (left) and naturally regenerated secondary forests (i.e. forests that are naturally regenerating after a significant disturbance such as a clearance for agriculture) (right). The numbers (1–6) refer to the six vanilla rows per site, in which each row contains one individual of each *Vanilla* species, following a random planting design. In cacao plantations, *Erythrina* was planted as the vanilla tutor tree between the cacao rows. In secondary forests, a mix of forest tree species was used as vanilla tutor trees. (C) Demonstration of a random planting design of the different *Vanilla* species across the six rows. The squares represent a vanilla plant (and its tutor tree), colored by *Vanilla* species.

(Kohlmann et al., 2010), consists of two national parks (Corcovado and Piedras Blancas National Park) connected by the Golfo Dulce Forest Reserve and a biological corridor that serve as buffer zones around the protected areas (SINAC, 2018), and is surrounded by a fragmented landscape of human settlements and agricultural lands. Annual precipitation ranges from 2500 to 6000 mm, and the average temperature is 25 °C (Holdridge, 1967; Kappelle, 2016). We studied the performance of six *Vanilla* species, of which two are commercially used species in Costa Rica: *Vanilla planifolia* and a disease-resistant hybrid ((*V. planifolia* x *V. pompona*) x *V. planifolia*). The other four species are fragrant *Vanilla* CWRs naturally occurring within ACOSA: *Vanilla hartii*, *V. odorata*, *V. pompona*, and *V. trigonocarpa*. Likewise *V. planifolia*, they belong to *Vanilla* sect. *Xanata* and can be seen as secondary gene pool CWRs, as previous hybridizations demonstrated the possibility of gene transfer (e.g. *V. planifolia* x *V. pompona*). All are native to the Neotropics, but with distinct distributions (Watteyn et al., 2020). Species can be distinguished via their leaves, stems, flowers and fruits (Soto Arenas and Dressler, 2010; Karremans et al., 2020).

2.2. Experimental design

The experimental design consisted of 12 field sites, of which six were located in secondary forests (SF) (i.e. forests that are naturally regenerating after a significant disturbance such as a clearance for agriculture) and six in cacao (*Theobroma cacao* L.) plantations (CP). The 12 sites, spread across ACOSA (Fig. 2A), were selected based on the availability of SFs and CPs, and farmers' willingness to cooperate with the study. In each of the sites, six individuals per *Vanilla* species were planted in June 2019, totaling 36 plants per site. One week before planting, eight-nodded vegetative cuttings of the two commercially used species (*V. planifolia* and hybrid) and the four CWRs were collected from, respectively, local plantations and wild populations present in ACOSA. Cuttings were sealed to avoid potential proliferation of fungal and bacterial contamination during transportation from the collection to the field site. Each site consisted of six rows, and each row contained one individual per species (Fig. 2B-C).

Vanilla being a climbing orchid, needs a so-called tutor tree. Following the traditional method, 1.70 m long *Erythrina* cuttings were planted as nitrogen-fixing tutor trees between the cacao rows in the CPs. No cacao trees were used as vanilla tutor trees as cacao flowers and thus fruits are being developed on cacao trees' trunks and branches (cauliflory), hence the presence of a vanilla vine growing around the branches would interfere with cacao production. CPs were between four and six years old, followed a traditional 3 m x 3 m planting design (3 m spacing between cacao trees in the same and adjacent rows) (Fig. 2B – cacao plantation), and were characterized by the presence of shade trees that also produce additional food commodities (not shown in figure). The shade provided by former trees allowed us to directly integrate the vanilla cuttings after tutor tree planting, whereas tutor trees are usually planted one year ahead if implemented on a cleared land. Within the SFs, we selected vanilla tutor trees among the tree species available in the field site. Selection was based on the following characteristics: (i) trees with a diameter at breast height (DBH) $\geq$ 5 cm and no peeling bark, and (ii) trees that allowed us to roughly generate 6 rows within the field site (Fig. 2B – secondary forest). The vanilla cuttings were planted by placing the lower three nodes onto the soil and covering it with onsite available organic material. The upper five nodes were tied up onto the tutor tree using biodegradable jute twine to avoid strangulation. The planting order of the different *Vanilla* species varied among the rows to avoid potential edge-effects (Fig. 2C).

2.3. Cultivation potential of Vanilla CWRs

2.3.1. Vanilla plant performance

2.3.1.1. Plant performance traits. For two consecutive years, we collected trait data related to the performance of the vanilla plants. More specifically, we recorded plant survival and measured variables related to plant vitality, growth and flowering during three periods, starting six months after planting (December 2019, June 2020 and December 2020). We selected these specific moments as they correspond with the dry (December) and rainy (June) season. An overview of the measured plant performance traits can be found in Table 1. Considering vanilla plants (at least of the commercially used species *V. planifolia* and the hybrid) generally start to flower three to four years after planting (Havkin--Frenkel and Belanger, 2018), we did not expect to observe any flowering within the timeframe of our study. However, flowers were detected at the end of 2020 (last monitoring period) and throughout 2021 for the CWR species *V. hartii*, *V. odorata*, and *V. pompona*, of which the number of inflorescences and flowers were recorded.

2.3.1.2. Site-specific environmental variables. We evaluated a number of site-specific environmental variables to better understand their potential effect on vanilla plant performance besides the effect of *Vanilla* species and land use system (detailed overview in Supporting Material A – Table S1). The variables measured at site level were elevation (m), slope (%), onsite tree species diversity, soil bacterial diversity and soil chemical properties (pH, acidity, saturated acidity, organic matter, macro- and micronutrients). The examined variables at vanilla plant level were relative photosynthetic active radiation, and tutor tree species, height and diameter at breast height.

For the variables related to the soil, 25 random bulk soil samples (0–10 cm depth) were collected from each site in 2019 and 2020. A

Table 1
Overview of the measured *Vanilla* plant performance traits related to plant survival, vitality (planted cuttings and newly developed shoots) and growth as well as the variables derived from the measured vitality and growth traits. All traits was measured during each of the three measurement periods: December 2019, June 2020, December 2020.

	Trait	Score / index
Survival		
	Plant survival[a]	(a) Dead (b) Alive
Vitality		
1. Planted cutting	General condition of the planted cutting[b]	(a) Necrosis (b) No necrosis
2. Newly developed shoot	Herbivore damage[c]	(a) Strong (b) Medium (c) Insignificant
	Pathogen presence[c]	(a) Strong (b) Medium (c) Insignificant
	Leaf discoloration[c]	(a) Strong (b) Medium (c) Insignificant
	Missing leaves	Count
	Missing shoot apices	Count
Growth		
	Roots	(a) Absent (b) Present
	New shoot	(a) Absent (b) Present
	Number of new shoots	Count
	Number of internodes per shoot	Count
	Internode length	cm
	Internode diameter	mm
	Leaf area[d]	cm^2
Derived variables		
	Percentage missing leaves (= # missing leaves / # internodes)	%
	Percentage missing shoot apices (= # missing shoot apices / # new shoots)	%
	Total shoot length (= sum of internode lengths per shoot)	cm
	Total vine length (= sum of internode lengths of all shoots)	cm
	Mean internode length	cm
	Mean internode diameter	mm
	Total leaf area	cm^2
	Mean leaf area	cm^2

[a] If we encountered the planted cutting in a very bad condition (e.g. nearly dead), but a new shoot was observed, the individual was marked as alive. [b] We ensured that the planted cuttings, at least visually, were in good health at the start of the experiment (i.e. absence of externally visible pathogens or herbivory, healthy colored leaves and stems), and measured the presence of necrosis during each of the three measurement periods to examine the vitality of the planted cuttings over time, as new shoots and roots are developed from these cuttings. [c] The variables herbivory damage, pathogen presence and leaf discoloration were scored using pictures as an example (Supporting Material A – Fig. S1). [d] The leaf area of six medium-sized leaves. Note: during the first measurement period, we removed three medium-sized leaves from each vanilla plant and measured their dimensions. The leaf area was calculated using Image 1.53 n (Schneider et al., 2012) and a linear regression model was built per species to predict leaf area with leaf length and width, allowing us to continue with non-destructive leaf area measurements.

composite soil sample was obtained by mixing the 25 samples per site, and large woody litter was removed from the soil samples. Air-dried samples were then sieved to be stored at 4 °C for analysis of soil chemical properties, and at − 20 °C for metabarcoding analysis of soil microbiota. The latter implied the extraction of DNA from 250 mg of soil sample, in triplicate, using a DNeasy PowerSoil Kit (Qiagen) and the MM400 homogenizer (Retsch GmbH, Haan, Germany), following the manufacturer's guidelines. The V4 region of bacterial 16S rRNA gene was amplified using the barcoded primers F515 and R806, as described previously by Caporaso et al. (2011). Libraries were sequenced on the Illumina NovaSeq 6000 using 2 × 250 bp paired reads, with 100,000 reads per sample (Novogene Corporation Inc., Sacramento, CA). Primer and adapter sequences were removed with Cutadapt (Martin, 2011), ASVs were inferred with DADA2 (R package DADA2, Callahan et al., 2016), and taxonomic assignment was performed with a naive Bayesian classifier (Wang et al., 2007) and the SILVA SSU r138.1 database (Quast et al., 2012). For alpha diversity analysis, inferred ASVs were pre-processed by (a) removing the ASVs classified as "Chloroplast", "Mitochondria", or "Eukaryota", and (b) filtering out the ASVs not assigned to Bacteria or Archaea. The filtered raw counts were normalized by scaling with ranked subsampling (Beule and Karlovsky, 2020). We calculated and compared diversity indices among site and land use system (R package phyloseq, McMurdie and Holmes, 2013; R package vegan, Oksanen et al., 2020), and identified dominant bacterial phyla of which their relative abundances significantly differed among sites (R package ALDEx2, Fernandes et al., 2013) (detailed methodology in Supplementary Material B – Text S1, Fig. S1-S2). From this, the relative abundance of these soil bacterial phyla as well as the soil bacterial diversity (Shannon-Wiener index) were added as predictor variables in subsequent vanilla plant performance models. Moreover, we applied a principal component analysis (PCA) with scaled variables (Legendre and Gallagher, 2001) to the dataset containing the soil chemical properties to examine potential correlations and reduce the dimensionality of this dataset (detailed methodology in Supplementary Material B – Text S1, Fig. S3). As a result, the first and second axis, respectively associated to soil acidity (pH, Fe, Ca, K, saturated acidity) and nutrient cycling (e.g. Mg, organic matter, P) were added as predictor variables ("soil pca1″ and "soil pca2″) in subsequent vanilla plant performance models.

The onsite tree species diversity was assessed by identifying and counting all trees with a DBH ≥ 5 cm, besides the trees used as vanilla tutor trees. Tree species were identified in the field with the help of two local botanists. In case of doubtful identification, leaves and branches (if possible also flowers and fruits) were collected, and species were identified using plant identification keys. Alpha diversity metrics were calculated and compared among site and land use system. Additionally, to explain potential effects of the vanilla tutor trees and on-site (shade) trees on vanilla plant performance, we collected functional tree trait data from the TRY (Kattge et al., 2020) and BIEN (Maitner et al., 2018) databases. For each species, we gathered information on the following available traits, which relate to nutrient cycling and nutritional quality, and may affect plant performance (Lavorel and Grigulis et al., 2012; Díaz et al., 2016): leaf carbon (C), leaf nitrogen (N), leaf phosphorous (P), specific leaf area (SLA), leaf dry matter content (LDMC), leaf thickness, lamina fracture toughness, leaf C:N ratio, leaf C:P ratio, leaf N:P ratio, and leaf lignin. The community-weighted means (CWM) of each of the functional tree traits as well as the functional tree diversity were calculated at site level (R package FD, Laliberté et al., 2014) (detailed methodology in Supplementary Material B – Text S1, Figs. S4–5). From this, the onsite (shade) tree species diversity (Shannon-Wiener index), the CMWs of the functional traits of the shade trees, and the functional trait values of the vanilla tutor trees were added as predictor variables in subsequent vanilla plant performance models.

2.3.1.3. Vanilla plant performance models. First, a factorial analysis of mixed data (FAMD) (R package FactoMineR, Lê et al., 2008; R package FactoExtra, Kassambara and Mundt, 2017) was used to reduce the complexity of the dataset encompassing the measured vitality and growth traits of the newly developed shoots. We examined potential correlations among the traits and analysed their contribution to the observed variation within the dataset (detailed methodology in Supplementary Material B – Text S2, Fig. S6-8). Based on the FAMD, axes 1 and 2 were used as proxies for, respectively, the growth and vitality of the newly developed shoots, in vanilla plant performance models. We then applied generalized additive mixed models (GAMMs) (R package mgcv) (Wood, 2017) to evaluate the effects of the predictor variables of interest, *Vanilla* species and land use system, as well as site-specific environmental variables (elevation, slope, relative light intensity, soil bacteria diversity, relative abundance of certain soil bacteria phyla, soil chemical properties pca1 and pca2, onsite tree species diversity, and functional tree traits of both vanilla tutor and shade trees), on plant survival, vitality of the cuttings, vitality of the shoots and growth. As such, we developed four models representing these vanilla plant performance traits: survival model, vitality cutting model, vitality shoot model, growth model. GAMMs allowed us to (i) account for the correlation structure in our data by including the random effect of site as well as of measurement period, and (ii) to model nonlinear relationships between response and predictor variables. We allowed the model fitting to penalize predictor variables (covariates) to 0 so that covariates weakly associated with the response variable (i.e. plant performance traits) could be completely removed from the model, resulting in model selection through joint penalization of multiple model terms (Wood and Augustin, 2002; Marra and Wood, 2011). To view the relationship between the predictor and explanatory variables, we plotted the partial response curves (R package mgcViz) (Fasiolo et al., 2020) showing the relationship of the partial residuals of the response variable on the linear predictor scale and the explanatory variables.

2.3.2. Aromatic profiles of Vanilla CWR pods

Two pods per *Vanilla* species (including the crop species *V. planifolia*, hybrid and CWRs; all belonging to the aromatic section *Xanata*) were collected for aromatic compound analysis. Pods of the hybrid were harvested from the same plantation that provided the vegetative cuttings, and pods of the CWRs were harvested from the wild populations from where we collected the cuttings (Supporting Material C – Table S1). No pods of *V. planifolia* could be harvested due to the absence of flowering *V. planifolia* plantations within our study area. As such, we used *V. planifolia* pods available at Instituto Tecnológico de Tuxtepec, where subsequent analysis was carried out. All pods were harvested just before complete maturation, i.e. nine months after pollination, as the aromatic compounds are mainly developed during the last months of fruit growth and accumulated as glycosides (Palama et al., 2009). The collected pods were freeze-dried to minimize possible enzymatic degradation, and stored at − 20 °C until analysis. We followed the methodology described in Pérez-Silva et al. (2021) to analyze the aromatic compounds via high-performance liquid chromatography (HPLC) analysis, using an Agilent 1260 Infinity II series apparatus equipped with a Diodo array detector HS (G7117C), Multicolumn Thermostat (G7116A), Quaternary Pump unit (G7111B) and Autosampler (G7129A). Separation and quantification were performed on ZORBAX Eclipse XDB-C18 column (250 × 4.6 mm i.d, 5 µm particle size). The mobile phase involved a mixture of the solvents: A (HPLC-grade water), B (HPLC-grade methanol) and C (10^{-2} M H_3PO_4). The column temperature was of 30 °C and the volume of injection was 10 µL. the detection of the components was made to 230 nm for vanillin, vanillyl alcohol, p-hydroxybenzyl alcohol (p-HB alcohol) y anisyl alcohol; 254 nm for vanillic acid and p-hydroxybenzoic acid (p-HB acid); and 280 nm for anisaldehyde and p-hydroxybenzaldehyde (p-HB). Solutions of the compounds (1–100 mg/l) were separately prepared in the mobile phase and injected into the HPLC-DAD system to build the calibration curve. For each species, the average contents of glucovanillin and aglycones released by hydrolysis were calculated, and the aglycones were grouped per family (vanillyl, p-hydroxybenzyl (pHB) and anisyl) (Table 2). The vanillin potential was determined by adding the content of free vanillin and 48.4 % (i.e., ratio of vanillin/glucovanillin molecular weights) of the content of glucovanillin directly determined by HPLC (without enzymatic hydrolysis) – the same protocol as described in Pérez-Silva et al. (2021) was applied. Finally, a PCA was carried out to visually explore the differences and similarities among the *Vanilla* species, based on the aromatic profiles derived from HPLC-DAD quantifications.

All statistical analyses were performed using R version 4.1.1.

3. Results

3.1. Vanilla plant performance

3.1.1. Comparison of plant performance among Vanilla species and land use systems

Although not significant, we observed a difference in plant survival (survival model, AIC = 373.26, Supporting Material D – Table S1) among the *Vanilla* species in the two land use systems. More specifically, the survival probability of *V. hartii* and *V. trigonocarpa* was slightly higher in SFs compared to CPs, while *V. planifolia* seemed to survive better in CPs (Fig. 3A). The other species (hybrid and CWRs *V. odorata* and *V. pompona*) had similar survival probabilities in both systems. The survival of the hybrid was the highest among all species, both in CPs and SFs. The vitality of the planted cuttings (vitality cutting model, AIC = 807.73, Supporting Material D – Table S2) of the hybrid and the CWRs was significantly higher compared to the crop species *V. planifolia* (Fig. 3B). The cuttings of *V. planifolia* showed the lowest vitality in SFs, whereas the vitality of the planted cuttings of the other species did not differ between the two land use systems. The vitality of the newly developed shoots (vitality shoot model, AIC = 2913.97, Supporting Material D – Table S3) of the hybrid and *V. pompona* was significantly higher compared to the other species (*V. planifolia* and CWRs *V. hartii*, *V. odorata* and *V. trigonocarpa*), and this in both land use systems (Fig. 3C). *Vanilla planifolia* had a lower shoot vitality in SFs compared to CPs, whereas the other species performed similar in both systems. The overall growth rate (growth model, AIC = 3612.5, Supporting Material D – Table S4) of the commercially used species (crop species *V. planifolia* and the hybrid) was higher compared to the four CWRs, but with differences between the two land use systems (Fig. 3D). *Vanilla planifolia*, the hybrid and *V. odorata* grew significantly better in CPs, while the growth of the other three CWRs was similar in CPs and SFs. The CWR *V. trigonocarpa* had the lowest growth rate in both land use systems.

Overall, *Vanilla planifolia* performed less in SFs compared to CPs, and had the lowest plant vitality of all species, while the hybrid and CWR *V. pompona* showed the highest plant vitality. The growth rate of the CWRs was lower compared to the crop species *V. planifolia* and the hybrid, but this difference was only significant in CPs. The hybrid showed the best overall plant performance in both land use systems, taking into account plant survival, vitality and growth. Finally, we also found that the cutting vitality significantly affected the vitality and growth of the newly developed shoots (Supporting Material D – Table S3-S4), whereby a high vitality of planted cuttings positively affected plant vitality and growth.

3.1.2. Effects of site-specific environmental variables on vanilla plant performance

Relative light intensity affected vanilla plant survival, vitality and growth, yet, the effect varied among *Vanilla* species (Fig. 4). The survival probability of the crop species *V. planifolia*, the hybrid as well as the CWRs *V. pompona* and *V. trigonocarpa* increased with light intensity, yet survival probabilities decreased again at light intensities above ± 40 %, except for *V. trigonocarpa*. There was no significant effect of light on the survival probability of the CWRs *V. hartii* and *V. odorata*, yet *V. hartii*'s survival seemed to slightly decrease with light intensity. Light negatively affected the vitality of *V. hartii* cuttings, while the cuttings of the hybrid and *V. odorata* performed best with light intensities between 20 % and 40 % and 15–25 %, respectively. *Vanilla planifolia* cuttings performed best between 20 % and 40 %, although not significant. Light positively affected both the vitality and growth of the shoots of *V. planifolia* and the hybrid, while it did not have a significant effect on the shoot vitality or growth of the CWRs.

The other site-specific variables had varied effects on plant performance (Supporting Material D - Fig. S1). Soil acidity ("Soil PCA1″) had a negative effect on plant survival, while soil organic matter ("Soil PCA2″ – not shown in figure) did not affect vanilla plant performance. Soil bacterial diversity had a positive effect on the vitality of the planted cuttings, and the relative abundance of certain soil bacterial phyla affected vanilla plant performance in different ways. Bacteroidota and Firmicutes positively affected plant survival, and Bacteroidota also had a positive effect on plant growth. Nitrospirota positively affected cutting vitality and plant growth, and Verrucomicrobiota had a positive effect on the vitality of both cuttings and shoots. Acidobacteriota and Actinocateriota positively affected vanilla plant growth, and there seemed to

Table 2
Mean contents of main phenolic and anisyl compounds analysed by HPLC expressed in g·100 g^{-1} dry weight in mature vanilla pods of the different *Vanilla* species and hybrids.

Vanilla spp.	Vanillin potential[a]	Vanillyl family			
		Glucovanillin	Vanillin[b]	Vanillic acid	Vanillyl alcohol
V. hartii	0.000	0.000	0.000	0.000	0.338 ± 0.011
V. helleri	0.861 ± 0.001	0.085 ± 0.001	0.820 ± 0.018	0.000	0.089 ± 0.003
Hybrid	4.654 ± 0.091	0.901 ± 0.003	4.218 ± 0.093	0.077 ± 0.002	0.143 ± 0.002
V. odorata	5.553 ± 0.078	7.861 ± 0.165	1.748 ± 0.036	0.088 ± 0.008	1.333 ± 0.081
V. planifolia	6.731 ± 0.046	13.748 ± 0.069	0.076 ± 0.002	0.182 ± 0.010	0.232 ± 0.006
V. pompona	0.000	0.000	0.000	0.000	0.000
V. trigonocarpa	0.022 ± 0.003	0.000	0.022 ± 0.003	0.128 ± 0.003	0.877 ± 0.006

Vanilla spp.	p-hydroxybenzyl family			Anisyl family		Total aromatic content
	pHB[c]	pHB acid[d]	pHB alcohol[e]	Anisyl alcohol	Anisyl acid	
V. hartii	0.000	0.000	0.757 ± 0.036	4.757 ± 0.195	0.000	5.851 ± 0.241
V. helleri	0.035 ± 0.004	0.025 ± 0.002	0.645 ± 0.023	0.193 ± 0.007	0.340 ± 0.016	3.155 ± 0.103
Hybrid	0.298 ± 0.024	0.032 ± 0.001	0.240 ± 0.008	0.112 ± 0.003	0.000	5.556 ± 0.131
V. odorata	0.015 ± 0.001	0.036 ± 0.004	0.328 ± 0.014	0.127 ± 0.003	0.000	7.480 ± 0.027
V. planifolia	0.219 ± 0.022	0.078 ± 0.006	1.007 ± 0.004	0.000	0.000	8.449 ± 0.005
V. pompona	0.000	0.000	1.570 ± 0.092	1.584 ± 0.011	0.000	2.189 ± 0.055
V. trigonocarpa	0.000	0.015 ± 0.005	2.167 ± 0.011	0.000	0.000	3.209 ± 0.002

[a]0.484 times the content of glucovanillin added to the free vanillin content. [b] Quantification after enzymatic hydrolysis. [c]pHB = 4-hydroxybenzaldehyde. [d]pHB acid = 4-hydroxybenzoic acid. [e]pHB alcohol = 4-hydroxybenzyl alcohol. Note: the high vanillin and low glucovanillin content in the pods of the hybrid was due to the high level of pod maturity when harvested.

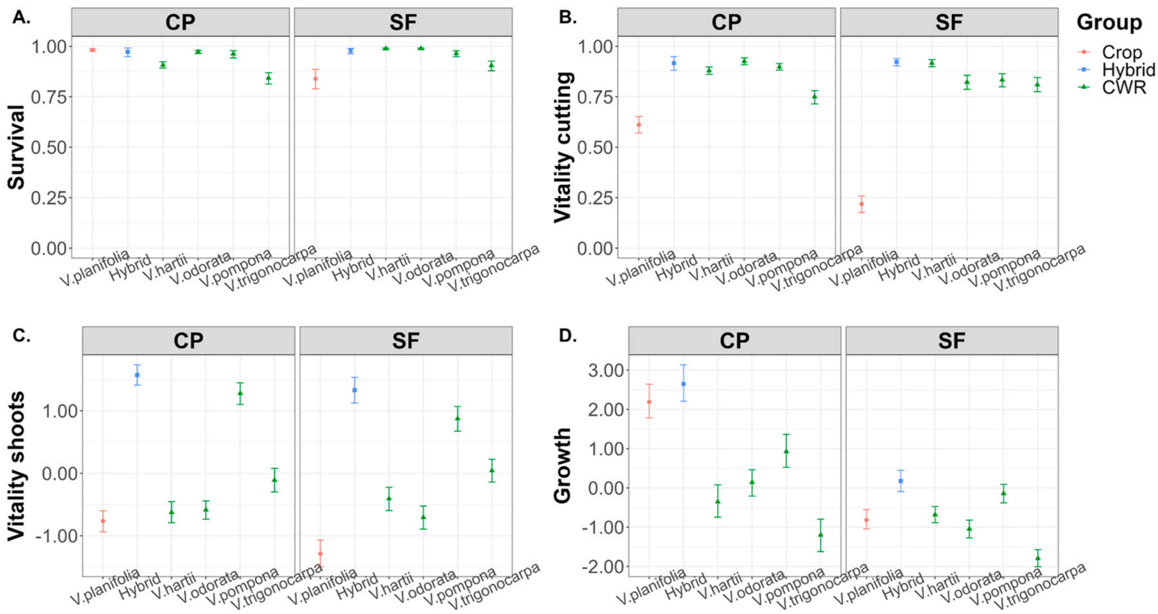

Fig. 3. Predicted effects of *Vanilla* species and land use system on (A) vanilla plant survival, (B) the vitality of the planted cuttings, (C) the vitality of the newly developed shoots, and (D) vanilla plant growth (i.e. growth of the newly developed shoots); grouped according to crop species, hybrid or CWR.

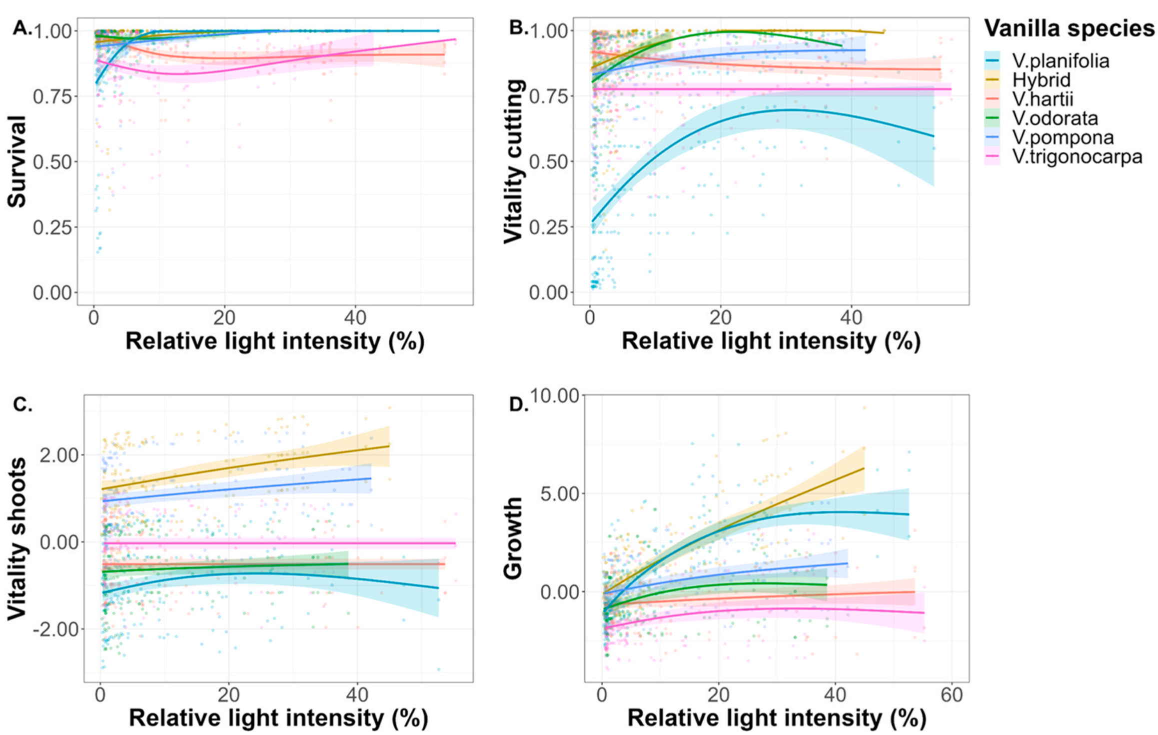

Fig. 4. Predicted effect of relative light intensity (%) on vanilla plant survival, vitality of the planted cutting, vitality of the newly developed shoots and vanilla plant growth, colored by *Vanilla* species.

exist an optimum Armatimonadota abundance for plant vitality. Among the functional traits of the vanilla tutor and shade trees, we observed significant effects of the leaf C:N ratio, SLA, and leaf toughness. In general, increasing SLA of both tutor and shade trees negatively affected plant survival and growth. Leaf toughness had a positive effect on the vitality and growth of the newly developed shoots, while we observed varied effects of leaf C:N ratio on vanilla plant performance. We observed a significant effect of the random effect terms site and measurement period, indicating that vanilla plant performance is possibly influenced by site- and period-specific variables that were not captured by our models.

3.1.3. Flowering of Vanilla species

Within the timeframe of this study, we were able to observe the first flowering period of some *Vanilla* species. More specifically, the CWRs *V. hartii*, *V. odorata* and *V. pompona* started to flower during the third measurement period (< 2 years after planting). The flowering *V. hartii* plants had on average 12.00 ± 4.95 flowers per plant (1.80 ± 0.84 inflorescences / plant), while flowering *V. odorata* and *V. pompona* plants had on average 12.70 ± 10.00 (2.00 ± 1.58 inflorescences/plant) and

20.70 ± 16.40 (2.86 ± 2.12 inflorescences / plant) flowers per plant, respectively.

3.2. Aromatic profiles of Vanilla CWR pods

The average contents of glucovanillin and aglycones released by hydrolysis (grouped per family: vanillyl, p-hydroxybenzyl (pHB) and anisyl) are given in Table 2. The first and second PCA axis explained 64.3 % of the total variation within the dataset (Supporting Material C - Fig. S1). The first axis was correlated with vanillin potential, pHB acid, vanillic acid on the one hand, and anisic alcohol on the other hand. The second axis was correlated with pHB alcohol and total pHB. *Vanilla pompona* and *V. hartii* were clearly separated at the left end of the first axis, as the pHB acid content and vanillin potential were below the detection threshold, whereas the vanillin-rich species, *V. planifolia*, *V. odorata* and the hybrid, were found at the very right end. The high anisyl alcohol content in *V. hartii* and *V. pompona* further explains their left-sided position along the first axis. The lower vanillin potential and pHB acid content of *V. helleri* and *V. trigonocarpa* explains their more centric position. The second axis mainly separated *V. trigonocarpa*, *V. planifolia* and *V. pompona* due their higher pHB alcohol content, with the latter found in all species but in different quantities. *Vanilla odorata* and the hybrid contained all compounds except for anisyl acid. Apart from vanillic acid, *Vanilla helleri* contained all of the examined compounds, yet, in rather small quantities.

4. Discussion

4.1. Cultivation potential of Vanilla CWRs

Based on our assessment of *Vanilla* CWR plant performance and aromatic profiles, we see potential in the integration of CWRs within the two studied land use systems (CPs and SFs) that may serve as vanilla cultivation areas surrounding natural forests possibly holding wild *Vanilla* populations. Corresponding planting designs should, however, take into account species-specific light intensity preferences, monitor the presence of soil microbiota and chemical properties, and select vanilla tutor and shade trees with optimal functional traits, amongst others. Furthermore, three *Vanilla* CWRs started to flower less than two years after planting, coinciding with the flowering period of their wild populations (personal observations). Since plants in commercial *V. planifolia* plantations generally start to flower three to four years after planting, this observed earliness (i.e., the display of early flowering phenotypes) could be a highly desirable trait for breeding programs, yet requires a more in-depth investigation.

4.1.1. Vanilla plant performance

The six *Vanilla* species had nearly similar survival probabilities in both land use systems. The hybrid showed the highest overall plant vitality and growth, and this in both CPs and SF, while *V. planifolia* was characterized by a rather low vitality, but high growth rates, and clearly performs inferior in SFs. The CWRs had a good overall vitality, and this in the two land use systems, but were characterized by lower growth rates compared to the commercially used species. The observed trends may be explained by past selections of fast-growing *V. planifolia* genotypes, which resulted in a reduced genetic variability in the cultivated *V. planifolia* lineages and plants being more susceptible to abiotic and biotic stressors (Bhai and Thomas, 2000; Bory et al., 2008; Pinaria et al., 2010; Adame-García et al., 2015). On the contrary, CWRs continue to evolve in the wild, developing traits to survive in changing environments (Dempewolf et al., 2017). The CWR *V. pompona* and its hybrid with *V. planifolia*, for example, are known to be highly resistant against fungal diseases and droughts (Theis and Jimenez, 1957; Grisoni and Nany, 2021). This resistance is linked to the formation of lignified hypodermal cell walls and the production of phenolic root exudates (Koyyappurath et al., 2015), and possibly explains the high vitality of *V. pompona* and the hybrid observed in our study. From our observations, we recommend to further evaluate species-specific resistance of *Vanilla* CWRs against (a)biotic stressors, especially stressors expected to occur under climate change, using controlled experiments that simulate extreme climate conditions and pathogen attacks, amongst others.

The performance of the different *Vanilla* species varied with light intensity. *Vanilla hartii* performed better with limited light availability, while the plant performance of the hybrid and *V. planifolia* was positively affected by light intensity, reaching an optimum between 20% and 50%, after which their performance decreased – as previously observed by Puthur (2005) and Díez et al. (2017) in *V. planifolia*. Although less pronounced, the plant performance of *V. pompona* and V. trigonocarpa was also positively affected by light, why the latter did not seem to affect *V. odorata*. The observed responses of the CWRs to light could be due to the fact that *V. hartii* and *V. odorata* naturally occur in the understory of old-growth forests, whereas *V. pompona* and *V. trigonocarpa* are found in secondary forests and even wetlands (Householder et al., 2010; Soto Arenas and Dressler, 2010; Watteyn et al., 2020). We only examined the effects of onsite light availability. To fully understand species-specific responses to light, a broader light spectrum should be considered.

Besides *Vanilla* species, land use system and light availability, we also observed certain effects of the additionally measured environmental variables on vanilla plant performance. Soil bacterial diversity positively affected vanilla plant survival, and the abundance of some bacterial phyla affected vanilla plant performance. Microorganisms play an important role in nutrient cycling, primary production, litter decomposition and climate regulation (Van Der Heijden et al., 2008; Delgado-Baquerizo et al., 2016), and they have been associated with plant health and growth (Garbeva et al., 2004; Chaparro et al., 2012; Harman and Uphoff, 2019; Bertola et al., 2021). For example, we observed a positive effect of the relative abundance of Bacteroidota, Firmicutes and Verrucomicrobiota – bacterial phyla that previously have been associated with vanilla disease-suppressive soils (Xiong et al., 2015, 2017) – on vanilla plant survival and vitality. Furthermore, Bacteroidota play an important role in soil mineralization (Fierer et al., 2007), and Nitrospirota, Actinobacteriota and Acidobacteriota have been recognized as plant growth-promoting bacteria (Kielak et al., 2016; Kalam et al., 2020; Yoneda et al., 2021), explaining the positive effect of these bacterial phyla on vanilla plant growth. Unfortunately, we were not able to analyse fungal diversity and composition within the timeframe of this study. Xiong et al., (2015, 2017) and Sandheep and Jisha (2014), however, showed that *Mortierella* and *Trichoderma* are pathogen suppressors, and a range of mycorrhizal fungi (e.g., *Ceratobasidium*, *Thanatephorus*, *Tulasnella*) have been associated with vanilla plant growth (Porras-Alfaro and Bayman, 2007; Flanagan and Mosquera-Espinosa, 2016; Johnson, 2021). We also observed a negative effect of soil acidity on vanilla plant survival, which is in accordance with past studies examining optimal soil properties and management for the vanilla crop (e.g., La et al., 1998; Osorio et al., 2014; Xiong et al., 2017). Some of the measured functional traits of both vanilla tutor and shade trees (e.g., leaf C:N ratio, SLA, leaf toughness) affected vanilla plant performance, which may be explained by the fact that tree functional traits have varied (indirect) effects on soil microbiota and nutrient cycling (Gillespie et al., 2021). Tree traits may also respond to light availability. For example, SLA decreases with light availability (Błasiak et al., 2021), which may explain the observed negative effect of SLA on vanilla plant performance, as light rather positively affected plant performance. As such, the selection of adequate vanilla tutor as well as shade trees may be a focal point in the design of vanilla agroforestry systems, as previously observed in cacao plantations (Sauvadet et al., 2019). We recommend to further examine potential feedback loops between soil chemical properties, functional tree traits and soil microbiota, jointly affecting vanilla plant performance, as this could greatly enhance our understanding of the functionality of vanilla agroforestry systems.

Lastly, we would like to elaborate on the positive effect of healthy vanilla cuttings on the vitality and growth of newly developed shoots, as it indicates the importance of acquiring healthy material when starting a vanilla plantation (see Azofeifa-Bolaños et al., 2019). We particularly observed a low vitality of *V. planifolia* cuttings. Although Grisoni and Nany (2021) recommended a *V. planifolia* variety ("Handa") as being highly resistant and with superior agronomic traits, and Azofeifa-Bolaños et al. (2014) established germplasm collections for the improvement of vanilla plant breeding in Costa Rica, the *V. planifolia* plants used in our study and derived from a local plantation did not seem to resemble the abovementioned features. We therefore emphasize the need to improve the exchange of healthy and certified vegetative material for commercial planting. Since our observations were based on visual assessments, we suggest to evaluate the presence of pathogens through molecular studies (e.g. presence of *Fusarium oxysporum* through 16S and ITS rDNA metabarcoding – Adame-García et al., 2015 and Carbajal-Valenzuela et al., 2022), especially prior to the exchange of material.

4.1.2. Aromatic profiles of Vanilla CWR pods

The crop species *V. planifolia*, the hybrid and the CWR *V. odorata* had the greatest vanillin potential. The high vanillin content of *V. planifolia* conforms its historical path as the widely used crop species. The vanillyl and pHB compound contents in the *V. planifolia* pods used in our study were higher compared to previous studies of P é rez Silva et al. (2021), endorsing the formerly recognized variability in the aromatic potential of this crop species. Although *V. odorata* is the paternal parent of *V. ^{x}tahitensis* – a species cultivated in Indonesia and with unique aromatic features (Brunschwig et al., 2017; Favre et al., 2022) – this is the first study that quantified the aromatic potential of *V. odorata* itself. Vanillyl, pHB and anisyl compounds were found in both the hybrid and *V. odorata*, indicating their potential for market integration and thus diversification. Moreover, our findings regarding the hybrid – widely cultivated in Costa Rica but not found on the international market (Havkin-Belanger et al., 2018) – correspond with former observations of Pérez-Silva et al. (2021). Anisyl alcohol was the major compound in both *V. hartii* and *V. pompona*, and is highly appreciated compound in specialty markets such as cosmetics, as previously recognized by Maruenda et al. (2013) and Pérez-Silva et al. (2021) for *V. pompona*. *Vanilla helleri* contained a high diversity of compounds but in small quantities, and *V. trigonocarpa* had the highest pHB alcohol content, explaining its rather fruity type odor.

Although our explorative study provided novel insights into the diversity of aromatic compounds present in *Vanilla* CWR pods, it had some limitations: (i) only two pods per *Vanilla* species were analysed, and (ii) pods were harvested from wild populations (for the CWRs) or plantations (V. planifolia, hybrid). To provide solid recommendations on the aromatic potential, and thus market potential, of *Vanilla* CWRs, more pods should be analysed and ideally be harvested from our experimental field sites (i.e. similar conditions to allow comparison among pods). Additional research should also quantify the aromatic compounds present in cured pods to fully capture the species' aromatic potential, using a combination of HPLC and gas chromatography-mass spectrum (GC-MS) analysis. Moreover, the HPLC chromatograms showed peaks that may correspond to non-aromatic fatty acids or other, non-identified aromatic compounds. We must recognize that the integration of CWRs into the market will only be possible through adaptations in international regulations, as the latter currently only allow the commercialization of *V. planifolia*, *V. pompona*, *V.* ^{x} *tahitensis* and the hybrid, and are solely qualifying vanilla pods by means of their vanillin potential.

4.2. Proposition of a cultivation design with Vanilla CWRs

We see potential in a combined cultivation of commercially used *Vanilla* species (*V. planifolia*, hybrid) and *Vanilla* CWRs within the evaluated land use systems (CP, SF), yet current systems will require some adaptations to account for species-specific light intensity preferences, to provide optimal soil microbial and chemical properties, and to integrate appropriate vanilla tutor trees. For example, the cultivation of a mix of *Vanilla* species in existing cacao plantations will require a spatial transformation of the cacao planting design (3 m $\times$ 3 m). We already noticed some competition for light between the cacao trees and the incorporated vanilla tutor trees (*Erythrina*), which may negatively affect crop management on the long-term. The specific light preferences of the different *Vanilla* species, together with cacao trees' light requirements, will entail a precise design and corresponding management (e.g., pruning). Regarding the secondary forests, we observed a couple of drawbacks, such as randomly spread vanilla tutor trees – typical for naturally regenerated forests (Zhang et al., 2020) – and the lack of suitable tutor tree branching patterns. These limitations prevent a proper guidance of vanilla vines from one tree to another (i.e. vanilla curtains) or within the same tree (i.e., up- and downwards coiling). Correct vine management is vital to facilitate manual pollination and harvest, as well as to ensure sufficient ventilation (Havkin-Frenkel and Belanger, 2018). We therefore recommend the following: (i) Implementation of so-called vanilla agroforests on fallow lands, whereby a mix of (shade) tree species are selected and distributed in accordance to *Vanilla* species-specific light preferences, and vanilla tutor trees are incorporated in the understory. Tutor trees should enable a proper vine management and possess favorable traits (e.g., low branching pattern, non-peeling bark, specific functional tree traits, amongst others), and could even provide additional products such as fruits or nuts (Osewold et al., 2022). (ii) Integration of vanilla in existing secondary forests, whereby vanilla tutor trees are planted inside the forest instead of using the onsite available trees as tutor trees. The available trees function as shade trees, and possibly need to be managed to enable optimal light conditions. The latter option is already implemented by some farmers (e.g., in Madagascar – Osewold et al., 2022). Yet, it may not be the preferred option, as the cultivation of crops within forests has received quite some criticism in the past, due to its negative effect on forest biodiversity and associated ecosystem services (Aerts et al., 2011; Martin et al., 2020). Finally, in both land use systems, soil properties should be carefully monitored over time to ensure optimal soil acidity and stimulate the presence of growth-promoting and disease-suppressive microbiota.

Following these conclusions, we propose the following cultivation design that integrates the six *Vanilla* species within the evaluated land use systems (Fig. 5):

Yet, the design vastly needs further evaluation in terms of feasibility. The following aspects related to management, production, as well as biodiversity should be examined, and ideally be compared with intensively managed vanilla monocultures: (i) Optimal light conditions and spatial distribution of *Vanilla* species and other (additional) crops. (ii) Calculations of yield, production costs and revenues associated with the cultivation of different *Vanilla* species, and in the proposed systems. This should include data on flowering patterns (e.g. flowering periods, number of inflorescences and flowers), fruit development (e.g., percentage of aborting or deformed fruits, fruit capacity per plant), market integration and prices, as well as costs related to manual pollination, harvest and management. For the latter, one should take into account the multiple flowering periods of certain *Vanilla* species and the potential lack of flowering synchronization among species. (iii) Evaluation of the functionality of the proposed systems not only in terms of productivity but also biodiversity and ecosystem functioning, to benefit farmers and nature alike (following the methodology of Wurz et al., 2022, who compared yield and biodiversity in fallow-derived or foret-derived vanilla agroforests of Madagascar).

Besides the need to further evaluate the feasibility of this approach, we need to recognize the certain threat for admixture between the wild *Vanilla* gene pool and vanilla cultivars – as has been observed with coffee (Aerts et al., 2013). Potential risks could, however, be minimized by only allowing the use of local species in the cultivation areas neighboring natural forests, thereby serving as a buffer with the areas that

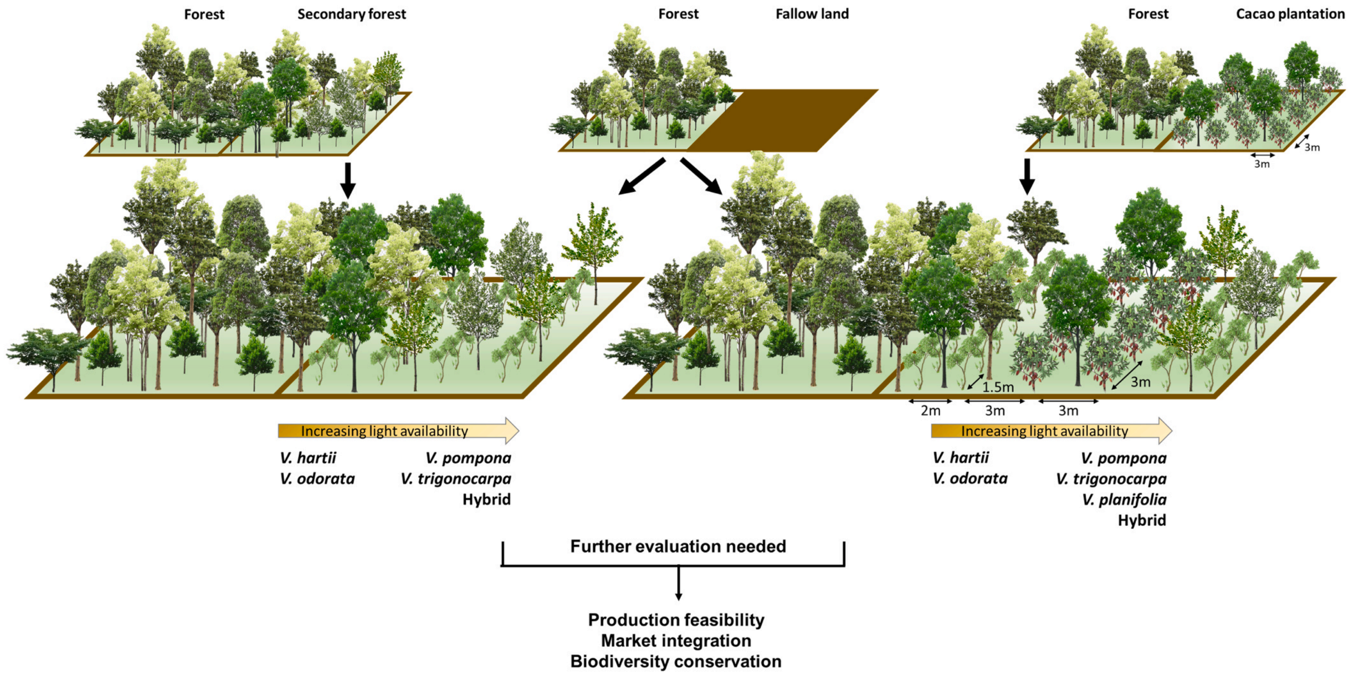

Fig. 5. Possible design for the integration of *Vanilla* CWRs within a production context, that should take into account species-specific preferences that allow optimal vanilla plant performance. For example, *V. hartii* and *V. odorata* seemed to perform better with limited light availability, hence, a habitat resembling old-growth forests. The other *Vanilla* species require higher light intensities and thus less dense canopies with a larger spatial distribution. The proposed designs are focusing on secondary forest (left), fallow land (middle) or existing agrosystems such as a cacao plantation (right), and definitely require further evaluation of production feasibility, market integration and biodiversity conservation. Forest refers to either old-growth or secondary forests adjacent to potential vanilla cultivation areas.

may include improved cultivars. Genotyping *Vanilla* populations will enhance our knowledge on existing genetic diversity present within the wild gene pool, and improve the development of appropriate in situ and *ex situ* conservation as well as cultivation schemes at local and regional scales.

CRediT authorship contribution statement

Charlotte Watteyn: Conceptualization, Methodology, Data collection and analysis, Writing – original draft, Writing – review & editing. **Bert Reubens**: Conceptualization, Methodology, Writing – review & editing. **José Bernal Azofeifa Bolaños**: Conceptualization, Methodology, Data collection and analysis, Writing – review & editing. **Frank Solano Campos**: Methodology, Data collection and analysis, Writing – review & editing. **Araceli P é rez Silva**: Methodology, Data collection and analysis, Writing – review & editing. **Adam Karremans**: Methodology, Writing – review & editing, Supervision. **Bart Muys**: Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data and codes used in our study have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.3ffbg79pt). The raw sequence files are publicly available through the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA898075, linked to BioSample accessions SAMN31597310-SAMN31597320.

Acknowledgments

This work was supported by the Doctoral Grant Strategic Basic Research (1S80820N) of the Research Foundation Flanders (FWO). Research and collection permits were provided by the Ministerio de Ambiente y Energía (MINAE-SINAC). We would like to give a special thanks to all the farm owners for letting us implement the experimental field sites on their lands, José Perez Fuentes and José Araya Orozco for their help with the fieldwork, Amelia Paniagua Vásquez for coordinating the analysis of the soil samples, and the Vaniclim lab of the Instituto Tecnológico de Tuxtepec for assisting with the aromatic profile analysis of the vanilla pods.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2023.126890.

References

Adame-García, J., Rodríguez-Guerra, R., Iglesias-Andreu, L.G., Ramos-Prado, J.M., Luna-Rodríguez, M., 2015. Molecular identification and pathogenic variation of Fusarium species isolated from Vanilla planifolia in Papantla Mexico. Bot. Sci. 93 (3), 669–678.

Aerts, R., Hundera, K., Berecha, G., Gijbels, P., Baeten, M., Van Mechelen, M., Hermy, M., Muys, B., Honnay, O., 2011. Semi-forest coffee cultivation and the conservation of Ethiopian Afromontane rainforest fragments. For. Ecol. Manag. 261 (6), 1034–1041.

Aerts, R., Berecha, G., Gijbels, P., Hundera, K., Van Glabeke, S., Vandepitte, K., Muys, B., Roldán-Ruiz, I., Honnay, O., 2013. Genetic variation and risks of introgression in the wild C offea arabica gene pool in south-western E thiopian montane rainforests. Evolut. Appl. 6 (2), 243–252.

Azofeifa-Bolaños, J.B., Paniagua-Vásquez, A., García-García, J.A., 2014. Importancia y desafíos de la conservación de Vanilla spp.(Orquidaceae) en Costa Rica. Agron. Mesoam. 25 (1), 189–202.

Azofeifa-Bolaños, J.B., Rivera-Coto, G., Paniagua-Vásquez, A., Cordero-Solórzano, R., Salas-Alvarado, E., 2019. Efecto de la desinfección de segmentos nodales sobre el rendimiento morfogenético de vitroplantas de Vanilla planifolia Andrews. Agron. Mesoam. 30 (1), 33–49.

Bertola, M., Ferrarini, A., Visioli, G., 2021. Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by-omics approaches: A perspective for the environment, food quality and human safety. Microorganisms 9 (7), 1400.

Beule, L., Karlovsky, P., 2020. Improved normalization of species count data in ecology by scaling with ranked subsampling (SRS): application to microbial communities. PeerJ 8, e9593.

Bhai, R.S., Thomas, J., 2000. Phytophthora rot–a new disease of vanilla (Vanilla planifolia Andrews) in India. J. Spices Aromat. Crops 9 (1), 73–75.

Błasiak, A., Węgiel, A., Łukowski, A., Sułkowski, S., Turski, M., 2021. The effects of tree and stand traits on the specific leaf area in managed scots pine forests of different ages. Forests 12 (4), 396.

Bory, S., Grisoni, M., Duval, M.F. and Besse, P., 2008. Biodiversity and preservation of vanilla: present state of knowledge. Genetic Resources and Crop Evolution, 55(4), pp.551–571.

Bramel, P., Frey, F. 2021. Global strategy for the conservation and use of Vanilla genetic resources. Global Crop Diversity Trust. Bonn, Germany.

Brunschwig, C., Collard, F.X., Lepers-Andrzejewski, S. and Raharivelomanana, P., 2017. Tahitian Vanilla (Vanilla× tahitensis): a Vanilla species with unique features. Active Ingredients from Aromatic and Medicinal Plants.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J.,. & Knight, R. , 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the national academy of sciences, 108(Supplement 1), 4516–4522.

Carbajal-Valenzuela, I.A., Muñoz-Sanchez, A.H., Hernández-Hernández, J., Barona-Gómez, F., Truong, C., Cibrian-Jaramillo, A., 2022. Microbial diversity in cultivated and feral vanilla vanilla planifolia orchids affected by stem and rot disease. Microb. Ecol. 84 (3), 821–833.

Chambers, A., Cibrián-Jaramillo, A., Karremans, A.P., Martinez, D.M., Hernandez-Hernandez, J., Brym, M., Resende Jr, M.F., Moloney, R., Sierra, S.N., Hasing, T., Alomia, Y.A., 2021. Genotyping-By-Sequencing diversity analysis of international Vanilla collections uncovers hidden diversity and enables plant improvement. Plant Sci. 311, 111019.

Chaparro, J.M., Sheflin, A.M., Manter, D.K., Vivanco, J.M., 2012. Manipulating the soil microbiome to increase soil health and plant fertility. Biol. Fertil. Soils 48 (5), 489–499.

Chen, Q., Li, W., Tan, L., Tian, F., 2021. Harnessing knowledge from maize and rice domestication for new crop breeding. Mol. Plant 14 (1), 9–26.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat. Commun. 7 (1), 1–8.

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., Guarino, L., 2017. Past and future use of wild relatives in crop breeding. Crop Sci. 57 (3), 1070–1082.

Díaz, S., Kattge, J., Cornelissen, J.H., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C., Colin Prentice, I., Garnier, E., 2016. The global spectrum of plant form and function. Nature 529 (7585), 167–171.

Díez, M.C., Moreno, F., Gantiva, E., 2017. Effects of light intensity on the morphology and CAM photosynthesis of Vanilla planifolia Andrews. Rev. Fac. Nac. De. Agron. Medellín 70 (1), 8023–8033.

Fasiolo, M., Nedellec, R., Goude, Y., Wood, S.N., 2020. Scalable visualization methods for modern generalized additive models. Journal of computational and Graphical Statistics 29 (1), 78–86.

Favre, F., Jourda, C., Grisoni, M., Piet, Q., Rivallan, R., Dijoux, J.B., Hascoat, J., Lepers-Andrzejewski, S., Besse, P., Charron, C., 2022. A genome-wide assessment of the genetic diversity, evolution and relationships with allied species of the clonally propagated crop Vanilla planifolia Jacks. ex Andrews. Genet. Resour. Crop Evol. 1–15.

Fernandes, A.D., Macklaim, J.M., Linn, T.G., Reid, G., Gloor, G.B., 2013. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PLOS One 8 (7), e67019.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88 (6), 1354–1364.

Flanagan, N.S., Mosquera-Espinosa, A.T., 2016. An integrated strategy for the conservation and sustainable use of native Vanilla species in Colombia. Lankesteriana 16 (2), 201–218.

Garbeva, P.V., Van Veen, J.A., Van Elsas, J.D., 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu. Rev. Phytopathol. 42, 243–270.

Gepts, P., 2004. Crop domestication as a long-term selection experiment. Plant Breed. Rev. 24 (2), 1–44.

Gillespie, L.M., Hättenschwiler, S., Milcu, A., Wambsganss, J., Shihan, A., Fromin, N., 2021. Tree species mixing affects soil microbial functioning indirectly via root and litter traits and soil parameters in European forests. Funct. Ecol. 35 (10), 2190–2204.

Goettsch, B., Urquiza-Haas, T., Koleff, P., Acevedo Gasman, F., Aguilar-Meléndez, A., Alavez, V., Alejandre-Iturbide, G., Aragón Cuevas, F., Azurdia Pérez, C., Carr, J.A., Castellanos-Morales, G., et al., 2021. Extinction risk of Mesoamerican crop wild relatives. Plants, People, Planet 3 (6), 775–795.

Grisoni, M., Nany, F., 2021. The beautiful hills: half a century of vanilla (Vanilla planifolia Jacks. ex Andrews) breeding in Madagascar. Genet. Resour. Crop Evol. 68 (5), 1691–1708.

Hänke, H., Barkmann, J., Blum, L., Franke, Y., Martin, D.A., Niens, J., Osen, K., Uruena, V., Witherspoon, S.A. and Wurz, A., 2018. Socio-economic, land use and value chain perspectives on vanilla farming in the SAVA Region (north-eastern Madagascar): The Diversity Turn Baseline Study (DTBS) (No. 1806).

Harlan, J.R., de Wet, J.M., 1971. Toward a rational classification of cultivated plants. Taxon 20 (4), 509–517.

Harman, G.E., Uphoff, N., 2019. Symbiotic root-endophytic soil microbes improve crop productivity and provide environmental benefits. Scientifica 2019.

Havkin-Frenkel, D., Belanger, F.C., 2018. Handbook of Vanilla Science and Technology. John Wiley & Sons.

Hodgkin, T. and Hajjar, R., 2007. Using crop wild relatives for crop improvement: trends and perspectives. Crop wild relative conservation and use, pp.535–548.

Holdridge, L.R., 1967. Life zone ecology. Life zone ecology., (rev. ed.).

Householder, E., Janovec, J., Mozambite, A.B., Maceda, J.H., Wells, J., Valega, R., Maruenda, H., Christenson, E., 2010. Diversity, natural history, and conservation of Vanilla (Orchidaceae) in Amazonian wetlands of Madre de Dios, Peru. J. Bot. Res. Inst. Tex. 227–243.

Johnson, L.J., Gónzalez-Chávez, M.D.C.A., Carrillo-González, R., Porras-Alfaro, A., Mueller, G.M., 2021. Vanilla aerial and terrestrial roots host rich communities of orchid mycorrhizal and ectomycorrhizal fungi. Plants, People, Planet 3 (5), 541–552.

Kalam, S., Basu, A., Ahmad, I., Sayyed, R.Z., El-Enshasy, H.A., Dailin, D.J., Suriani, N.L., 2020. Recent understanding of soil acidobacteria and their ecological significance: a critical review. Front. Microbiol. 2712.

Kappelle, M., 2016. *Costa Rican Ecosystems*. University of Chicago Press.

Karremans, A.P., Chinchilla, I.F., Rojas-Alvarado, G., Cedeño-Fonseca, M., Alexander, D., Léotard, G., 2020. A reappraisal of Neotropical Vanilla. With a note on taxonomic inflation and the importance of alpha taxonomy in biological studies. Lankesteriana 20 (3), 395.

Kassambara, A. and Mundt, F., 2017. "Package 'factoextra'." Extract and visualize the results of multivariate data analyses.

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Tautenhahn, S., Werner, G.D., Aakala, T., Abedi, M., Acosta, A.T., 2020. TRY plant trait database–enhanced coverage and open access. Glob. Change Biol. 26 (1), 119–188.

Khan, A.W., Garg, V., Roorkiwal, M., Golicz, A.A., Edwards, D., Varshney, R.K., 2020. Super-pangenome by integrating the wild side of a species for accelerated crop improvement. Trends Plant Sci. 25 (2), 148–158.

Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., Van Veen, J.A., Kuramae, E.E., 2016. The ecology of Acidobacteria: moving beyond genes and genomes. Front. Microbiol. 7, 744.

Kohlmann, B., Roderus, D., Elle, O., Solís, Á., Soto, X., Russo, R., 2010. Biodiversity conservation in Costa Rica: a correspondence analysis between identified biodiversity hotspots (Araceae, Arecaceae, Bromeliaceae, and Scarabaeinae) and conservation priority life zones. Rev. Mex. De. Biodivers. 81 (2), 511–559.

Koyyappurath, S., Conéjéro, G., Dijoux, J.B., Lapeyre-Montès, F., Jade, K., Chiroleu, F., Gatineau, F., Verdeil, J.L., Besse, P., Grisoni, M., 2015. Differential responses of vanilla accessions to root rot and colonization by fusarium oxysporum f. sp. radicis-vanillae. Front. Plant Sci. 6, 1125.

La, C., Dian, L., Shumei, T., Shaoruo, Z., 1998. Nutritive characteristics of vanilla. Chin. J. Trop. Crops 2, 55–64.

Laliberté, E., Legendre, P., Shipley, B. and Laliberté, M.E., 2014. Package 'FD'. Measuring functional diversity from multiple traits, and other tools for functional ecology, pp.1–0.

Lavorel, S., Grigulis, K., 2012. How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. J. Ecol. 100 (1), 128–140.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 1–18.

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129 (2), 271–280.

Maitner, B.S., Boyle, B., Casler, N., Condit, R., Donoghue, J., Durán, S.M., Guaderrama, D., Hinchliff, C.E., Jørgensen, P.M., Kraft, N.J., McGill, B., 2018. The bien r package: a tool to access the Botanical Information and Ecology Network (BIEN) database. Methods Ecol. Evol. 9 (2), 373–379.

Marra, G., Wood, S.N., 2011. Practical variable selection for generalized additive models. Comput. Stat. Data Anal. 55 (7), 2372–2387.

Martin, D.A., Osen, K., Grass, I., Hölscher, D., Tscharntke, T., Wurz, A., Kreft, H., 2020. Land-use history determines ecosystem services and conservation value in tropical agroforestry. Conserv. Lett. 13 (5), e12740.

Martin, D.A., Wurz, A., Osen, K., Grass, I., Hölscher, D., Rabemanantsoa, T., Tscharntke, T., Kreft, H., 2021. Shade-tree rehabilitation in vanilla agroforests is yield neutral and may translate into landscape-scale canopy cover gains. Ecosystems 24 (5), 1253–1267.

Martin, D.A., Andrianisaina, F., Fulgence, T.R., Osen, K., Rakotomalala, A.A.N.A., Raveloaritiana, E., Soazafy, M.R., Wurz, A., Andriafanomezantsoa, R., Andriamaniraka, H., Andrianarimisa, A., Barkmann, J., Dröge, S., Grass, I., Guerrero-Ramirez, N., Hänke, H., Hölscher, D., Rakouth, B., Ranarijaona, H.L.T., and Kreft, H., 2022. Land-use trajectories for sustainable land system transformations: Identifying leverage points in a global biodiversity hotspot. Proceedings of the National Academy of Sciences, 119(7).

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J. 17 (1), 10–12. https://doi.org/10.14806/ej.17.1.200.

Maruenda, H., del Lujan Vico, M., Householder, J.E., Janovec, J.P., Cañari, C., Naka, A., Gonzalez, A.E., 2013. Exploration of Vanilla pompona from the Peruvian Amazon as a potential source of vanilla essence: Quantification of phenolics by HPLC-DAD. Food Chem. 138 (1), 161–167.

Maxted, N., Ford-Lloyd, B.V., Jury, S., Kell, S., Scholten, M., 2006. Towards a definition of a crop wild relative. Biodivers. Conserv. 15 (8), 2673–2685.

Maxted, N., Scholten, M., Codd, R., Ford-Lloyd, B., 2007. Creation and use of a national inventory of crop wild relatives. Biol. Conserv. 140 (1–2), 142–159.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS One 8 (4), e61217.

Meilleur, B.A., Hodgkin, T., 2004. In situ conservation of crop wild relatives: status and trends. Biodivers. Conserv. 13 (4), 663–684.

Meyer, R.S., Purugganan, M.D., 2013. Evolution of crop species: genetics of domestication and diversification. Nat. Rev. Genet. 14 (12), 840–852.

Oksanen, F.Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan Mc.Glinn, Peter R. Minchin, R.B. O.'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs and Helene Wagner, 2020. vegan: Community Ecology Package. R package version 2.5–7. https://CRAN.R-project.org/package=vegan.

Osewold, J., Korol, Y., Osen, K., Soazafy, M.R., Rabemanantsoa, T., Martin, D.A., Wurz, A., Hölscher, D., 2022. Support trees in vanilla agroforests of Madagascar: diversity, composition and origin. Agrofor. Syst. https://doi.org/10.1007/s10457-022-00733-y.

Osorio, A.I., Osorio Vega, N.W., Díez, M.C., Moreno, F.H., 2014. Nutrient status and vegetative growth of Vanilla planifolia Jacks plants as affected by fertilization and organic substrate composition. Acta Agron. 63 (4), 326–334.

Osterhoudt, S.R., 2017. Vanilla Landscapes: Meaning, Memory, and the Cultivation of Place in Madagascar. New York Botanical Garden, New York.

Palama, T.L., Khatib, A., Choi, Y.H., Payet, B., Fock, I., Verpoorte, R., Kodja, H., 2009. Metabolic changes in different developmental stages of Vanilla planifolia pods. J. Agric. Food Chem. 57 (17), 7651–7658.

Pérez-Silva, A., Nicolás-García, M., Petit, T., Dijoux, J.B., de los Ángeles Vivar-Vera, M., Besse, P., Grisoni, M., 2021. Quantification of the aromatic potential of ripe fruit of Vanilla planifolia (Orchidaceae) and several of its closely and distantly related species and hybrids. Eur. Food Res. Technol. 247 (6), 1489–1499.

Pinaria, A.G., Liew, E.C.Y., Burgess, L.W., 2010. Fusarium species associated with vanilla stem rot in Indonesia. Australas. Plant Pathol. 39 (2), 176–183.

Porras-Alfaro, A., Bayman, P., 2007. Mycorrhizal fungi of Vanilla: diversity, specificity and effects on seed germination and plant growth. Mycologia 99 (4), 510–525.

Puthur, J., 2005. Influence of light intensity on growth and crop productivity of Vanilla planifolia Andr. General and Applied. Plant Physiol. 31 (3–4), 215–224.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596.

Redden, R.J., Yadav, S.S., Maxted, N., Dulloo, M.E., Guarino, L., Smith, P., 2015. Crop Wild Relatives and Climate Change. John Wiley & Sons,.

Ross-Ibarra, J., Morrell, P.L., Gaut, B.S., 2007. Plant domestication, a unique opportunity to identify the genetic basis of adaptation. Proc. Natl. Acad. Sci. USA 104 (SUPPL 1), 8641–8648.

Sandheep, R.A., Jisha, M.S., 2014. Screening and identification of potential Trichoderma sp. against soil borne pathogens of vanilla (Vanilla planifolia). Indian J. Agric. Res. 48 (6), 459–464.

Sauvadet, M., Van den Meersche, K., Allinne, C., Gay, F., de Melo Virginio Filho, E., Chauvat, M., Becquer, T., Tixier, P., Harmand, J.M., 2019. Shade trees have higher impact on soil nutrient availability and food web in organic than conventional coffee agroforestry. Sci. Total Environ. 649, 1065–1074.

Schlüter, P.M., Arenas, M.A.S., Harris, S.A., 2007. Genetic variation in Vanilla planifolia (Orchidaceae). Econ. Bot. 61 (4), 328–336.

Schneider, C., Rasband, W., Eliceiri, K., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089.

SINAC. 2018. Plan estratégico 2018–2025 Programa nacional corredores biológicos de Costa Rica. http://enbcr.go.cr/sites/default/files/sinac_2018_planestrategico_programa_nacional_de_corredores_biologicos_costa_rica.pdf (accessed 25 October 2020).

Soto Arenas, M.A., Dressler, R.L., 2010. A revision of the Mexican and Central American species of Vanilla Plumier ex Miller with a characterization of their ITS region of the nuclear ribosomal DNA. Lankesteriana 9 (3), 285–354.

Tanksley, S.D., McCouch, S.R., 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277 (5329), 1063–1066.

Theis, T., Jimenez, F.A., 1957. A vanilla hybrid resistant to Fusarium root rot. Phytopathology 47 (10), 579–581.

Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11 (3), 296–310.

Van Heerwaarden, J., Doebley, J., Briggs, W.H., Glaubitz, J.C., Goodman, M.M., Gonzalez, J.D.J.S., Ross-Ibarra, J., 2011. Genetic signals of origin, spread, and introgression in a large sample of maize landraces. Proc. Natl. Acad. Sci. USA 108 (3), 1088–1092.

Vincent, H., Wiersema, J., Kell, S., Fielder, H., Dobbie, S., Castañeda-Álvarez, N.P., Guarino, L., Eastwood, R., León, B., Maxted, N., 2013. A prioritized crop wild relative inventory to help underpin global food security. Biol. Conserv. 167, 265–275.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73 (16), 5261–5267.

Watteyn, C., Fremout, T., Karremans, A.P., Huarcaya, R.P., Azofeifa Bolanos, J.B., Reubens, B., Muys, B., 2020. Vanilla distribution modeling for conservation and sustainable cultivation in a joint land sparing/sharing concept. Ecosphere 11 (3), e03056.

Wood, S., 2017. Package "mgcv": mixed GAM computation vehicle with GCV/AIC/REML smoothness estimation. Version.

Wood, S.N., Augustin, N.H., 2002. GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. Ecol. Model. 157 (2–3), 157–177.

Wurz, A., Tscharntke, T., Martin, D.A., Osen, K., Rakotomalala, A.A.N.A., Raveloaritiana, E., Andrianisaina, F., Dröge, S., Fulgence, T.R., Soazafy, M.R., Andriafanomezantsoa, R., Andrianarimisa, A., Babarezoto, F.S., Barkmann, J., Hänke, H., Hölscher, D., Kreft, H., Rakouth, B., Guerrero-Ramírez, N.R., Grass, I., 2022. Win-win opportunities combining high yields with high multi-taxa biodiversity in tropical agroforestry. Nat. Commun. 13 (1) (1).

Xiong, W., Abraham, P.E., Li, Z., Pan, C., Hettich, R.L., 2015. Microbial metaproteomics for characterizing the range of metabolic functions and activities of human gut microbiota. Proteomics 15 (20), 3424–3438.

Xiong, W., Guo, S., Jousset, A., Zhao, Q., Wu, H., Li, R., Kowalchuk, G.A., Shen, Q., 2017. Bio-fertilizer application induces soil suppressiveness against Fusarium wilt disease by reshaping the soil microbiome. Soil Biol. Biochem. 114, 238–247.

Yoneda, Y., Yamamoto, K., Makino, A., Tanaka, Y., Meng, X.Y., Hashimoto, J., Shin-Ya, K., Satoh, N., Fujie, M., Toyama, T., Mori, K., 2021. Novel plant-associated acidobacteria promotes growth of common floating aquatic plants, duckweeds. Microorganisms 9 (6), 1133.

Yumurtaci, A., 2015. Utilization of wild relatives of wheat, barley, maize and oat in developing abiotic and biotic stress tolerant new varieties. Emir. J. Food Agric. 01–23.

Zeder, M.A., Emshwiller, E., Smith, B.D., Bradley, D.G., 2006. Documenting domestication: the intersection of genetics and archaeology. Trends Genet. 22 (3), 139–155.

Zhang, L., Dong, L., Liu, Q., Liu, Z., 2020. Spatial patterns and interspecific associations during natural regeneration in three types of secondary forest in the central part of the Greater Khingan Mountains, Heilongjiang Province, China. Forests 11 (2), 152.