Influence of farm management and fermentation on cocoa bean quality: Disentangling the driving factors of quality aspects in *Theobroma cacao* L. from Peruvian Amazonia

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Meinen Eltern &
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SUMMARY

Cocoa, a globally important cash crop, is appreciated by a growing number of people in all parts of the world as ingredient in chocolate or other cocoa derived food products. In past decades, consumers have shown higher preference for healthier products resulting in increased demand for cocoa products with higher cocoa content with the benefit of reducing sugar consumption and to take advantage of the inherent health benefits of cocoa. Also, consumer awareness is leaning towards traceability of product origin together with sustainable farming practices and working conditions in the crop growing countries. Therefore, chocolate should no longer only taste good, but also fulfil the consumer's demand for beneficial traits and for sustainable and ecological responsible farming practices. Due to these changing trends, the production process of chocolate, to guarantee a high-quality final product, is under review with a new focus on upstream steps along the cocoa value chain such as selection of cacao genetic material, the farming conditions, crop growing techniques, post-harvest treatments, shipment and storage.

This thesis aims at better understanding of the relevant drivers of cocoa quality during the initial steps of the cocoa value chain. These are the selection of cacao varieties, the cultivation and the fermentation of cacao. The studies included in this thesis are based on in-field assessments and experimental fermentations that were carried out in one of the major cacao-growing regions of Peru, around the city of Tocache, San Martín. The project was conducted in cooperation with the United Nations Office on Drugs and Crime (UNODC). The particular focus is on the following parameters: (1) Influence of environmental conditions and farm management on cocoa quality; (2) Influence of different pulp pre-conditioning treatments on the fermentation process and resulting raw cocoa bean quality; (3) Influence of clone type and fermentation vessel material on the fermentation process and resulting raw cocoa bean quality. Based on the scientific results, this thesis provides suggestions for practical applications. These are described in the synthesis part of this work.

To study the impact of environment and farm management on cocoa bean quality, the relationships were analyzed between plant diversity and crop quantity (yield, fruit set, fruit size), pathogen incidence (*Moniliophthora perniciosa*, *Moniliophthora roreri*, *Phytophthora* spp.), and the profile of selected secondary compounds (methylxanthines and polyphenols) in seeds of 48 cacao trees from different farms in the study region. In addition, the impact of soil parameters, fertilization and pruning on the same variables were investigated. The study revealed a complex relationship between biodiversity effects in cacao agroforestry systems and farm management techniques on cocoa bean quality. A strong positive correlation was found between the diversity of herb and shrub layer and the number of healthy, ripe fruits observed in the respective trees. Furthermore, a greater diversity

of herb and shrub layer proved to influence the degree of fungal infestation of cacao fruits and trees. This could partly be explained by the presence of plant characteristic contents of polyphenols and methylxanthines, both types of compounds also being indicators of final cocoa bean quality. In contrast, diversity and abundance of the shade tree layer showed a negative effect on yield parameters. Adding fertilizer to ensure sufficient nutrient supply was generally beneficial to the yield and the resistance to pathogens. Further, a shade management with an open shade tree layer together with shape and phytosanitary pruning results in an improved resistance to pathogens and increased yield. Overall, this study provides evidence for the importance of biodiversity and plantation management on product yield, disease outbreak, and secondary metabolism of cacao. In summary, the study is one of the first that shows how farming conditions influence cocoa bean quality.

Fermentation of cacao seeds is a crucial step for the formation of aroma precursors which transform into the cocoa specific aromas during the subsequent roasting process. Therefore, fermentation plays a pivotal role for the quality of the final product. To ensure adequate fermentation of fresh cacao seeds, the process should be adapted to both genotype and environmental conditions. Pulp-preconditioning may be an option to influence the fermentation process, but different techniques have not been studied nor compared comprehensively in particular for Trinitario clones cultivated in Peru. In this study, the impact of three pulp pre-conditioning methods (i. e., pod storage, pulp drainage and inoculation with starter cultures) was analyzed on the course of the fermentation process and related cocoa bean quality. 16 experimental fermentations with a defined clone mixture in 252 L wooden boxes were conducted. The data confirmed that treatments lead to different quantities of free amino acids and polyphenols which cause variations of aroma profiles and possible health benefits of the cocoa beans. In particular, removal of pulp resulted in reduced and pod storage in increased bitter and astringent taste of cocoa liquor produced from the cocoa beans. These findings will enable cacao farmers and cooperatives to adjust their product quality in line with customers' and consumers' preferences engaging easily implementable pre-conditioning techniques.

Apart from fermentation techniques, the influence of genetic traits of cacao seeds on the fermentation process was studied. 49 pilot-scale single clone fermentations (27 L) of eight Trinitario fine and flavor clones and one Forastero cultivar were measured and analyzed. As additional parameter, boxes with and without polystyrene insulation were included into the analyses. A further investigational set-up of 41 micro-fermentations with clone mixtures was carried out in boxes made of four different timber types. The aim was to explore possible effects of vessel material on the course of fermentation. Single clone fermentations showed a high similarity between most clones, with only two clones showing significantly different patterns in terms of temperature profile and fermentation degree respectively. As justified by the few differences found among the different

clones, it can be recommended to farmers and cacao cooperatives to ferment clones as a mixture. Insulation of boxes caused a less strong temperature increase between 48 and 96 h of fermentation—presumably due to reduced inoculation and subsequent aeration of the fermentation mass. These results make it inadvisable to insulate fermentation boxes.

As far as known, this study is the first that reports a significant effect of the timber type on temperature development, degree of fermentation, and the quantity of free amino acids in cacao seeds. Despite promising effects of two of the timber types compared in this study, none of the four types seemed to be optimal in respect to availability of timber, suitability for box construction, and fermentation. Hence, further research on appropriate wood types to standardize fermentation boxes for commercial scale is required.

In summary, this study clearly shows that relevant quality aspects in cocoa beans are determined at the very first steps of the value chain such as cacao farming, selection of genetic material, and fermentation conditions. This highlights the pivotal importance of small-holder farmers and their cooperatives with respect to their influence on the final product quality. Consequently, chocolate companies would benefit from a stronger involvement of smallholders into the production process and consideration of the entire value chain from “farm to chocolate bar”. The practical application of novel insights obtained during this study, like the positive effect of diverse agroforestry systems, the potential of pulp pre-conditioning, and the search for appropriate timber for box fermentations, would be a step toward to more reliable chocolate quality. A tight cooperation with cacao farmers on these measures will certainly contribute to produce healthier, more biodiversity-friendly, and socially responsible produced chocolate.

ZUSAMMENFASSUNG

Kakao ist eine wichtige Weltwirtschaftspflanze, die in Form von Schokolade oder anderen Kakaoprodukten von zunehmend mehr Menschen in allen Teilen der Welt geschätzt wird. In den letzten Jahrzehnten haben Verbraucher eine zunehmende Präferenz für gesündere Produkte entwickelt, was zu einer steigenden Nachfrage nach Kakaoprodukten mit höherem Kakaogehalt geführt hat, zum einen, um den Zuckerkonsum zu senken und zum anderen um die gesundheitlichen Vorteile von Kakao zu nutzen. Das Bewusstsein der Verbraucher hinsichtlich der Rückverfolgbarkeit von Produkten sowie bezüglich nachhaltiger landwirtschaftlicher Anbaumethoden und Arbeitsbedingungen in den Anbauländern hat zudem stark zugenommen. Daher sollte Schokolade nicht mehr nur gut schmecken, sondern auch der Forderung des Verbrauchers nach gesundheitsförderlichen Eigenschaften und nach nachhaltigen und ökologisch verantwortlichen landwirtschaftlichen Praktiken nachkommen. Aufgrund dieser sich ändernden Trends wird neben dem Produktionsprozess von Schokolade zur Gewährleistung eines qualitativ hochwertigen Endprodukts, zunehmend ein neuer Schwerpunkt auf vorgelagerte Schritte entlang der Kakao-Wertschöpfungskette gelegt. Dies betrifft zum Beispiel die Auswahl von genetischem Pflanzenmaterial von Kakao, die Anbaubedingungen und -techniken, Nacherntebehandlungen, Transport und Lagerung.

Ziel dieser Arbeit ist es, die relevanten Einflussfaktoren der Kakaoqualität in den ersten Schritten der Kakao-Wertschöpfungskette besser zu verstehen. Diese sind die Auswahl der Kakaosorten sowie der Anbau und die Fermentation von Kakao. Die in dieser Arbeit enthaltenen Studien basieren auf Felduntersuchungen und experimentellen Fermentationen, die in einem der wichtigsten Kakaoanbaugebiete Perus in der Nähe der Stadt Tocache in der Region San Martín durchgeführt wurden. Das Projekt wurde in Zusammenarbeit mit dem „Büro der Vereinten Nationen für Drogen- und Verbrechensbekämpfung“ (UNODC) durchgeführt. Der besondere Schwerpunkt liegt auf folgenden Parametern: (1) Einfluss der Umweltbedingungen und der Betriebsführung auf die Kakaoqualität; (2) Einfluss verschiedener Fruchtfleischvorbehandlungen auf den Fermentationsprozess und die daraus resultierende Kakaoqualität; (3) Einfluss des Klontyps und des Fermentationsgefäßmaterials auf den Fermentationsprozess und die daraus resultierende Kakaoqualität. Aus den wissenschaftlichen Ergebnissen dieser Arbeit werden Vorschläge für praktische Anwendungen abgeleitet. Diese sind im Syntheseteil der Arbeit beschrieben.

Um die Auswirkungen von Umweltparametern und Plantagenmanagement auf die Kakaoqualität zu untersuchen, wurden die Zusammenhänge zwischen Pflanzenvielfalt und Erntemenge (Ertrag, Fruchtansatz, Fruchtgröße), Pathogenbefall (*Moniliophthora perniciosa*, *Moniliophthora roreri*,

Phytophthora spp.) sowie das Profil ausgewählter sekundärer Pflanzenstoffe (Methylxanthine und Polyphenole) in Samen von 48 Kakaobäumen auf verschiedenen Kakaofarmen in der Untersuchungsregion analysiert. Darüber hinaus wurde der Einfluss von Bodenparametern, Düngung und Baumschnitt auf diese Variablen untersucht. Diese Studie zeigte einen komplexen Zusammenhang zwischen den Auswirkungen der biologischen Vielfalt in Kakao-Agroforstsystemen und der Betriebsführung auf die resultierende Kakaoqualität. So bestand eine signifikante, positive Korrelation zwischen der Vielfalt der Kraut- und Strauchschicht und der Anzahl gesunder, reifer Früchte an den untersuchten Kakaobäumen. Darüber hinaus zeigte sich, dass eine größere Vielfalt in der Kraut- und Strauchschicht den Grad des Pilzbefalls von Kakaofrüchten und -bäumen beeinflusst. Dies konnte teilweise durch das Vorhandensein von pflanzencharakteristischen Mengen an Polyphenolen und Methylxanthinen erklärt werden, wobei beide Verbindungen auch Indikatoren für die Kakaoqualität sind. Im Gegensatz dazu wirkten sich Diversität und Abundanz der Schattenbaumschicht negativ auf die Ertragsmenge aus. Dünger zur Sicherstellung einer ausreichenden Nährstoffversorgung war im Allgemeinen vorteilhaft für den Ertrag und die Resistenz gegen Krankheitserreger. Darüber hinaus führt ein Beschattungsmanagement mit einer offenen Baumkronenschicht zusammen mit Formschnitt und phytosanitärem Schnitt zu einer verbesserten Resistenz gegen Krankheitserreger und einem erhöhten Ertrag. Insgesamt liefert diese Studie somit Belege für die Bedeutung der biologischen Vielfalt und der Betriebsführung in Bezug auf den Produktertrag, den Krankheitsbefall und den Sekundärstoffwechsel von Kakao. Zusammenfassend ist diese Studie eine der ersten, die zeigt, wie die landwirtschaftlichen Bedingungen die Qualität von Rohkakao beeinflussen.

Die Fermentation von Kakaosamen ist ein entscheidender Schritt für die Bildung von Aromavorstufen, die während des anschließenden Röstprozesses in kakaospezifische Aromen umgewandelt werden. Daher spielt die Fermentation eine entscheidende Rolle für die Qualität des Endprodukts. Um eine wirkungsvolle Fermentation von frischen Kakaosamen sicherzustellen, sollte das Verfahren sowohl an den Genotyp als auch an die Umweltbedingungen angepasst werden. Die Vorbehandlung von Kakaofruchtfleisch kann dabei eine Option sein, um den Fermentationsprozess zu beeinflussen, allerdings wurden unterschiedliche Techniken insbesondere für peruanische Trinitario-Klone bisher weder untersucht noch umfassend verglichen. In dieser Studie wurde der Einfluss von drei Methoden zur Vorbehandlung von Kakaofruchtfleisch (Lagerung von Kakaofrüchten, Abpressen des Fruchtfleisches und Inokulation mit Starterkulturen) auf den Verlauf des Fermentationsprozesses und die damit verbundene Qualität von Kakaobohnen untersucht. Es wurden 16 experimentelle Fermentationen mit einer definierten Klonmischung in 252 L Holzkisten durchgeführt. Unsere Daten bestätigten, dass die Vorbehandlungen des Fruchtfleisches zu unterschiedlichen Mengen an freien Aminosäuren und Polyphenolen im Rohkakao führen, was zu

einer Veränderung des Aromaprofils und möglichen gesundheitlichen Wirkungen der Kakaobohnen führt. Insbesondere die Reduzierung des Fruchtfleisches durch Abpressen bewirkte einen weniger bitteren und adstringierenden Geschmack der Kakaomasse, während die Lagerung von Kakaofrüchten diese Geschmacksqualitäten verstärkte. Diese Ergebnisse ermöglichen es Kakaobauern und Genossenschaften, ihre Produktqualität mit leicht umsetzbaren Vorbehandlungstechniken an die Vorgaben von Kunden und Verbrauchern anzupassen.

Neben Fermentationstechniken wurde der Einfluss genetischer Merkmale von Kakaosamen auf den Fermentationsprozess untersucht. 49 Einzelklon-Mikrofermentationen (27 L) von acht Trinitario-Edelkakaoklonen und einer Forastero-Sorte wurden analysiert. Als zusätzlicher Parameter wurden Boxen mit und ohne Polystyrolisolierung in die Analysen einbezogen. Des Weiteren wurden 41 Mikrofermentationen mit Klonmischungen in unterschiedlichen Kisten durchgeführt, die aus vier verschiedenen Holzarten bestanden. Ziel dieses Ansatzes war es, mögliche Auswirkungen des Gefäßmaterials auf den Fermentationsverlauf zu untersuchen. Die Einzelklonfermentationen zeigten eine hohe Ähnlichkeit zwischen den meisten Klonen auf, wobei nur zwei Klone signifikant unterschiedliche Muster zeigten, zum einen hinsichtlich des Temperaturprofils und zum anderen in Bezug auf den Fermentationsgrad. Aufgrund der wenigen Unterschiede zwischen den verschiedenen Klonen kann den Landwirten und Kakao-Genossenschaften empfohlen werden, diese Klone gemeinsam zu fermentieren. Die Isolierung von Kisten verursachte einen weniger starken Temperaturanstieg zwischen 48 und 96 Stunden der Fermentation - vermutlich wegen einer verringerten Inokulation und Belüftung der Fermentationsmasse. Diese Ergebnisse machen es nicht empfehlenswert, Fermentationsboxen zu isolieren.

Nach aktuellem Forschungsstand ist diese Studie die erste, die einen signifikanten Einfluss unterschiedlicher Holzarten auf die Temperaturentwicklung während der Fermentation sowie den Fermentationsgrad und die Menge an freien Aminosäuren von Kakaosamen nachweisen konnte. Trotz vielversprechender Auswirkungen von zwei der in dieser Studie verglichenen Holzarten war jedoch keine der vier Arten hinsichtlich der Holzverfügbarkeit, der Eignung für den Kistenbau und der Fermentation optimal. Daher sind weitere Untersuchungen zu geeigneten Holzarten für eine Standardisierung von Fermentationsboxen im kommerziellen Maßstab erforderlich.

Zusammenfassend zeigt diese Studie deutlich, dass relevante Qualitätsaspekte von Kakao in den ersten Schritten der Wertschöpfungskette, wie Kakaoanbau, Auswahl von genetischem Material und Fermentationsbedingungen, bestimmt werden. Dies unterstreicht die zentrale Bedeutung der Kleinbauern und ihrer Genossenschaften für die Qualität des Endprodukts. Folglich würden Schokoladenunternehmen von einer stärkeren Einbeziehung der Kleinbauern in den Produktionsprozess und von der Berücksichtigung der gesamten Wertschöpfungskette von der „Farm

zur Schokoladentafel“ profitieren. Die praktische Anwendung der neuartigen Erkenntnisse aus dieser Arbeit, wie die positive Wirkung verschiedener Agroforstsysteme, das Potenzial der Fruchtfleischvorbehandlung und die Suche nach Holz mit vorteilhaften Eigenschaften für die Kistenfermentation, wäre ein Schritt in Richtung zuverlässigerer Qualität von Schokoladenprodukten. Eine enge Zusammenarbeit mit den Kakaobauern bei diesen Maßnahmen würde sicherlich dazu beitragen, gesündere, ökologisch vielfältigere und sozial verantwortlich hergestellte Schokolade zu produzieren.

RESUMEN

El cacao, un cultivo comercial de importancia mundial, es apreciado como ingrediente del chocolate u otros productos alimenticios derivados del cacao por un número cada vez mayor de personas en todas partes del mundo. En las últimas décadas, los consumidores han mostrado una preferencia creciente por productos más saludables, lo que ha dado como resultado una mayor demanda de productos de cacao con mayor porcentaje de cacao para reducir el consumo de azúcar y aprovechar de los beneficios para la salud inherente en el cacao. Además, la conciencia del consumidor se inclina hacia la trazabilidad del origen del producto junto con prácticas agrícolas y condiciones de trabajo sostenibles en los países productores del cultivo. Por lo tanto, el chocolate ya no solo debe tener buen sabor, sino también satisfacer la demanda del consumidor de características beneficiosas y prácticas agrícolas sostenibles y ecológicamente responsables. Debido a estas nuevas tendencias, el proceso de producción del chocolate, para garantizar un producto final de alta calidad, se está revisando con un nuevo enfoque en los pasos iniciales a lo largo de la cadena de valor del cacao, como la selección del material genético del cacao, las condiciones ambientales, prácticas agrícolas, tratamientos poscosecha, transporte y almacenamiento.

Esta tesis tiene como objetivo destacar los impulsores relevantes de la calidad del cacao durante los pasos iniciales de la cadena de valor del cacao. Estos son la selección de variedades de cacao, el cultivo y la fermentación del cacao. Los estudios de esta tesis se basan en evaluaciones de campo y fermentaciones experimentales que se llevaron a cabo en una de las principales regiones productoras de cacao del Perú, alrededor de la ciudad de Tocache, San Martín. El proyecto se llevó a cabo en cooperación con la “Oficina de las Naciones Unidas contra la Droga y el Delito” (UNODC). El enfoque particular está en los siguientes parámetros: (1) influencia de las condiciones ambientales y el manejo de la finca en la calidad del cacao; (2) influencia de los diferentes tratamientos de acondicionamiento previo de la pulpa en el proceso de fermentación y la calidad del cacao resultante; (3) influencia del tipo de clon y del material de los cajones en el proceso de fermentación y la calidad del cacao resultante. Con base en los resultados científicos, esta tesis proporciona sugerencias para aplicaciones prácticas, de los cuales se describen en la parte de síntesis de este trabajo.

Para estudiar el impacto del medio ambiente y del manejo de la finca sobre la calidad de los granos de cacao, analizamos las relaciones entre la diversidad de plantas y del rendimiento (rendimiento, cuajado, tamaño de frutos), la incidencia de patógenos (*Moniliophthora perniciosa, Moniliophthora roreri*, *Phytophthora* spp.) y el perfil de compuestos secundarios seleccionados (metilxantinas y polifenoles) en semillas de 48 árboles de cacao de diferentes fincas de la región de estudio. Además,

se investigó el impacto de los parámetros del suelo, de la fertilización y de la poda sobre las mismas variables. Nuestro estudio reveló una relación compleja entre los efectos de la biodiversidad en los sistemas agroforestales de cacao y las técnicas de manejo de fincas sobre la calidad del cacao resultante. Encontramos fuertes correlaciones positivas entre la diversidad de la capa de hierbas y arbustos y el número de frutos maduros sanos observados en los árboles estudiados. Además, se demostró que una mayor diversidad de la capa de hierbas y arbustos influye en el grado de infestación fúngica de los frutos y árboles de cacao. Esto podría explicarse en parte por el contenido de polifenol y metilxantinas característicos de la planta, siendo ambos tipos de compuestos indicadores de la calidad final del cacao. En cambio, la diversidad y abundancia de la capa de árboles de sombra mostró un efecto negativo en el rendimiento del producto. Agregar fertilizante para asegurar un suministro suficiente de nutrientes fue generalmente beneficioso para el rendimiento y la resistencia a los patógenos. Además, un manejo de la sombra con una capa de árboles de sombra abierta junto con la poda de formación y poda fitosanitaria resultó en una mejor resistencia a los patógenos y un mayor rendimiento. En general, nuestro estudio muestra la importancia de la biodiversidad y del manejo de las plantaciones en el rendimiento del producto, el brote de plagas y el metabolismo secundario del cacao. En resumen, este estudio es uno de los primeros que muestra cómo las condiciones de cultivo se traducen en la calidad final del cacao.

La fermentación de semillas de cacao es un paso muy importante para la formación de precursores de aroma que se transforman en aromas específicos del cacao durante el proceso de tostado posterior. Por tanto, la fermentación juega un papel fundamental para la calidad del producto final. Para asegurar una fermentación adecuada de las semillas frescas de cacao, el proceso debe adaptarse tanto al genotipo como a las condiciones ambientales. El preacondicionamiento de la pulpa puede ser una opción para influir en el proceso de fermentación, pero no se han estudiado ni comparado de manera exhaustiva diferentes técnicas, en particular para los clones Trinitario en Perú. En este estudio, se examinó el impacto de tres métodos de preacondicionamiento de la pulpa (es decir, almacenamiento de las mazorcas, drenaje de la pulpa e inoculación con cultivos iniciadores) en el curso del proceso de fermentación y la calidad del grano de cacao relacionado. Se realizaron 16 fermentaciones experimentales con una mezcla de clones definida en cajones de madera de 252 L. Nuestros datos confirmaron que los tratamientos dan lugar a diferentes cantidades de aminoácidos libres y polifenoles en los granos de cacao que se traducen en variaciones de los perfiles aromáticos y posibles beneficios para la salud. En particular, reducir la pulpa dio como resultado un sabor menos amargo y astringente en el licor de cacao, mientras que el almacenamiento de las mazorcas de cacao lo aumentó. Nuestros hallazgos permitirán a los productores de cacao y a las cooperativas ajustar la calidad de sus productos de acuerdo con las preferencias de los clientes y consumidores mediante técnicas de preacondicionamiento fáciles de implementar.

Además de las técnicas de fermentación, se estudió la influencia de los rasgos genéticos de las semillas de cacao en el proceso de fermentación. Se analizaron 49 micro-fermentaciones de clones a escala piloto (27 L) de ocho clones finos y de sabor Trinitario y un cultivar Forastero. Como parámetro adicional, incluimos cajones con y sin aislamiento de poliestireno en los análisis. Se llevó a cabo una nueva investigación de 41 microfermentaciones con mezclas de clones en cajones hechas de cuatro tipos de madera diferentes. El objetivo era explorar los posibles efectos del material de los cajones en el curso de la fermentación. Las fermentaciones de un solo clon confirmaron una alta similitud entre la mayoría de los clones, con solo dos clones mostrando patrones significativamente diferentes en términos de perfil de temperatura durante la fermentación, respectivamente, del grado de fermentación. Justificado por las pocas diferencias encontradas entre los diferentes clones fermentados, se puede recomendar a los agricultores y las cooperativas de cacao fermentar conjuntamente estos clones. El aislamiento de los cajones provocó un aumento de temperatura menos fuerte entre las 48 y las 96 h de fermentación, presumiblemente debido a la inoculación reducida y la posterior aireación de la masa de fermentación. Según nuestros resultados, no se recomendaría el aislamiento de los cajones de fermentación.

Hasta donde sabemos, este estudio es el primero que encuentra un efecto significativo del tipo de madera de los cajones sobre el desarrollo de la temperatura, el grado de fermentación y la cantidad de aminoácidos libres en las semillas de cacao. A pesar de los efectos prometedores de dos de los tipos de madera comparados en este estudio, ninguno de los cuatro tipos parecía ser óptimo en cuanto a disponibilidad de la madera, idoneidad para la construcción de cajones y fermentación. Por lo tanto, se requiere más investigación sobre los tipos de madera apropiados para estandarizar los cajones de fermentación a escala comercial.

En resumen, este estudio muestra claramente que algunos aspectos de calidad relevantes en el cacao se determinan en los primeros pasos de la cadena de valor, como el cultivo del cacao, la selección de material genético y las condiciones de fermentación. Esto destaca la importancia fundamental de los pequeños agricultores y sus cooperativas con respecto a su influencia en la calidad del producto final. En consecuencia, las empresas de chocolate se beneficiarían de una mayor participación de los pequeños agricultores en el proceso de producción y de la consideración de toda la cadena de valor, desde la “chacra hasta la barra de chocolate”. La aplicación práctica de los conocimientos novedosos obtenidos durante este estudio, como el efecto positivo de diversos sistemas agroforestales, el potencial del preacondicionamiento de la pulpa y la búsqueda de una madera adecuada para la fermentación en cajones, sería un paso hacia la calidad de chocolate más confiable. Una intensa cooperación con los productores de cacao en estas medidas contribuirá sin duda a producir un chocolate más saludable, más respetuoso con la biodiversidad y producido socialmente responsable.

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1 INTRODUCTION

Cacao is one of the most important cash crops that is cultivated mainly in developing and emerging countries close to the equator (BEG, 2017) and processed mainly in industrialized countries in temperate and subtropical latitudes (LIEBEREI AND REISDORFF, 2012). In the past, there was only a weak direct connection between cacao growers on the one hand and cocoa processing companies on the other hand as many intermediate traders were involved. Therefore, commercial quality criteria were usually claimed for raw cocoa beans that had already been fermented and dried. During the last 20 years, a novel awareness has developed that quality of cocoa-based products, e.g., chocolate, depends on the manufacturing process of the entire production chain involving cacao varieties, farming conditions, and post-harvest treatments (SCHWAN AND WHEALS, 2004).

Today, owing to the increasing awareness of consumers regarding higher product and ingredient transparency (OPARA AND MAZAUD, 2001), an improved understanding of the underlying mechanisms of cacao quality aspects becomes more important. In addition, the ongoing trend to produce chocolate with higher cocoa percentage makes it necessary to improve the flavor of cocoa beans and chocolate itself. As cocoa beans and chocolate have also become functional foods with certain health benefits (SCHWAN AND WHEALS, 2004), it is economically pivotal to control the drivers that influence quality parameters to maintain or develop desired compounds.

Despite a growing body of investigations during the last decades, especially in the field of general fermentation procedures and theories (reviewed in DE VUYST AND WECKX, 2016), various aspects of cocoa bean quality remain unclear. In particular, practical fermentation studies with applicable methods for farmers, which include also fermentation protocols for single cacao clones, are still lacking. Also, the effects of farm management conditions on the quality traits of cocoa beans have so far scarcely been under study (see ZUG et al., 2019).

1.1 Objectives and outline of this study

The overall objective of this study was to better understand the underlying mechanisms that influence the quality of raw cocoa beans during the production steps of cacao cultivation and fermentation. The studies were conducted in the department of San Martín, Peru, over a period of four consecutive years from 2011 to 2014. This thesis comprises five chapters which present and summarize the results. In particular, the influence of farm management conditions (biodiversity of plants in the herb, shrub and shade tree layer, pest incidence, soil conditions, fertilization, pesticide use and pruning) on cacao trees in terms of yield and seed quality was evaluated. Furthermore, the fermentation process in Peruvian cacao was studied by exploring a) pulp pre-conditioning methods

with Trinitario fine and flavor cacao mixtures and b) differences in the fermentation process among different fine and flavor cacaos along with possible effects of the fermentation vessels used. Based on the findings, local farmers can be supported to optimize their raw cocoa bean quality and possibly receive a higher revenue for their product.

The first chapter of this thesis provides general insights about the background of the studies, including the cacao tree, its cultivation, cacao varieties, distribution, propagation, pest and diseases, postharvest treatments, cacao flavors as well as the process of chocolate making and the cocoa market. The study was carried out within the framework of a regional project conducted by UNODC. Therefore, a brief overview on the project's objectives as well as a description of the study sites are given.

The second chapter of this study analyzes the effects of cacao farm management parameters in correlation with the cacao yield and seed quality. In particular, the interplay of plant diversity with pathogen incidence (*Moniliophthora perniciosa, Moniliophthora roreri, Phytophthora* spp.), crop yield (yield per ha and per tree, fruit set, and fruit size), and with the profile of selected secondary compounds (methylxanthines and polyphenols) in seeds of 48 cacao trees of 14 farms in Peruvian Amazonia was disentangled. This chapter provides evidence for a tight relation of biodiversity on cacao plantations, pathogen incidence, and the secondary metabolism of cacao trees.

The third chapter aims at the improvement of cacao fermentation in Peruvian Amazonia through different techniques of pulp pre-conditioning easily applicable by farmers. It was explored if pod storage, pulp drainage, and inoculation with starter cultures had an impact on the fermentation process, the contents of free amino acids and polyphenols as well as on the sensory characteristics of a typical Trinitario clone mixture. The study found an array of potential adaptations of the common direct box fermentation which may be used to optimize cacao quality by pulp pre-conditioning treatments.

The fourth chapter provides a comparison of the fermentation process of different Trinitario clones using single-clone fermentations. The study furthermore involves an analysis of the possible impact of fermentation vessel material on the fermentation process. It was revealed that seeds of the respective clones ferment mainly similarly. Furthermore, the material of the fermentation vessel influenced the fermentation quality.

In the fifth chapter, the other chapters are summarized in a synthesis, put into a larger context and jointly discussed.

Overall, the study aimed to answer the following superordinate study questions:

1. Do biodiversity and interactions with farm management and soil conditions have an impact on cacao yield, pathogen incidence, and plant secondary compounds?
2. How do pulp-preconditioning treatments prior to fermentation influence the course of fermentation and thus the quality and sensory traits of the raw cocoa beans?
3. Do genetic traits of the seed material along with the fermentation vessel properties influence the course of fermentation?

1.2 *Theobroma cacao* L.: An overview about cacao

1.2.1 Cacao morphology

Theobroma cacao L. naturally grows as a tropical understory tree up to a latitude of approx. 20° north and south of the equator. Cacao trees can grow up to 15 meters in height in natural forests in South America (FRANKE, 1994; LIEBEREI et al., 2010). The cacao tree has a tap root that grows approx. 1 to 2 meters long. The side roots mainly grow within the upper soil layers, up to 30 cm deep and up to 5 to 6 meters long (FRANKE, 1994). Naturally, the cacao tree has a characteristic tiered habitus. After a phase of orthotropic growth, the terminal bud divides into a so-called "jorquette" where 3 to 5 branches emerge with a plagiotropic growth pattern, the so called "fan-branches". Underneath the jorquette a new scion, the chupon, develops that becomes a new orthotope shoot (Fig. 1a). Every 1 to 1.5 years, a new jorquette is formed (LIEBEREI AND REISDOFF, 2012).

Figure 1: **a** Cacao tree with jorquette and chupon; **b** cacao buds and flowers in flower cushion; **c** unripe cacao fruit and "cherelle wilt" of young cacao pods.

The evergreen leaves of the cacao tree are stalked and between 15 to 30 cm long and up to 15 cm wide with an oval shape and a tip. Young cacao leaves often have a reddish to yellow coloring. As they do not yet possess full photosynthetic capacity, they are supplied with assimilates by adult leaves. In cacao, the leaf area of a tree can be related with its productivity (BALASIMHA et al., 1985). Cacao trees have cauliflorous and ramiflorous flowering and consequently fruit set at stem and branches (LIEBEREI AND REISDORFF, 2012). They start flowering and fruiting in meristematic flower cushions that are formed around the leaf axils at a tree age of 3 to 6 years, depending on the variety and the propagation technique. On average 30.000 to 50.000 flowers of approx. 1 cm in diameter are developed per tree and year (LIEBEREI et al., 2010). Parts of the cacao flower are fivefold with a green to reddish calyx, white-yellowish petals with an inner red stripe, as well as fertile white stamens and infertile, nectar rich staminodes (Fig. 1b). The five-chambered superior, coeno-syncarpous ovary has

a conical, yellowish style with a divided stigma. In each of the fruit compartments two rows of ovules develop. Trees flower and fruit all year round with usually one main crop and a second crop depending on the rainfall regime, but also on the availability of nutrients and adequate temperatures (LIEBEREI AND REISDORFF, 2012; LIEBEREI et al., 2010). Under dry or cool conditions, flowering can be entirely absent (ALVIM, 1984). Bud to flower development takes approx. 30 days. Pollination takes place in the early morning hours. Overall, only 0.5 to 5 percent of the cacao flowers become pollinated. The stamina are wrapped by the petals, which is the reason why a self-fertilization is impossible (LIEBEREI AND REISDORFF, 2012; LIEBEREI et al., 2010). Main pollinators are Ceratopogonidae (biting midges), mainly of the genera *Forcipomyia* and *Lasioshelea,* as well as different thrips and aphids (LIEBEREI et al., 2010). Furthermore, due to the so-called physiological condition "cherelle wilt", young cacao pods (fruits of cacao), "the cherelles", die off (MELNICK, 2016, Fig. 1c). Hence, up to 75 percent of the fruit set is lost and only 20 to 80 fruits per tree and year reach maturity. In cacao, there are self-compatible and incompatible varieties. Incompatible varieties always need another tree as a pollen donator. This self-incompatibility is based on the rejection of the male gamete by the female egg cell if both have the same incompatibility allele, which impedes the gamete from fusing with the egg cell (COPE, 1962). Self-incompatibility is often found in Trinitario varieties and Upper Amazon Forastero. However, the Trinitarios can often only be pollinated by self-fertile varieties whereas the latter can fertilize one another (WOOD AND LASS, 1989). Cacao fruits (pods) can be botanically classified as berries that take 120 to 180 days for ripening. Fruit color of ripe cacao pods range from yellow to dark purple with many coloration types depending on the genotype (LIEBEREI AND REISDORFF, 2012). Fruit sizes vary from 15 to 50 cm depending on the variety, nutrient availability, and precipitation. Unripe fruits have five compartments due to the partition walls of the originally septated ovary inside. During the ripening process, however, the septa become mucous hence the seeds embedded in the pulp lie in a single fruit cavity (LIEBEREI AND REISDORFF, 2012). The fruits usually contain between 20 and 50, 1.5 to 2.5 cm long, cream-white to purple-colored seeds, depending on the variety. They are arranged in a central axile placentation around a central spindle, that is also called placenta or "middle rip". The seeds consist of two strongly folded germ layers (cotyledons) and the embryo axis with radicle. They are surrounded by a testa (seed shell) that merged together with the endocarp part of the pulp (LIEBEREI AND REISDORFF, 2012). Cacao seeds are recalcitrant meaning that the germination starts directly after ripening with no seed dormancy. Cacao possesses an epigaic germination, the cotyledons develop above ground (LIEBEREI et al., 2010).

1.2.2 Cacao varieties and taxonomy

The cacao tree (*Theobroma cacao* L.) originates from the eastern slopes of the Andes in South America (MOTAMAYOR et al., 2002; ZHANG et al., 2006), where also the greatest genetic variability, great morphological admixture as well as disease resistance can be found (BOZA et al., 2014). Today,

it belongs to the family of the Malvaceae. Within the genus *Theobroma*, 22 species are known (CUATRECASAS, 1964). Besides *Theobroma cacao*, *Theobroma bicolor* Humb. et Bonpl. and *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. are cultivated in South and Middle America only, but mainly for local use. Hence, *Theobroma cacao* is so far the only species of worldwide economic importance (LIEBEREI AND REISDORFF, 2012).

Based on morphogenetic traits and geographical origins, two main genetic cacao groups "Criollo" (span.: "native") and "Forastero" (span.: "stranger") were traditionally defined (CHEESMAN, 1944). Additionally, a third group that comprises hybrids of "Criollo" and "Forastero" was named "Trinitario" (CHEESMAN, 1944). Parallel research described two subspecies of *T. cacao, T. cacao* L. ssp. *cacao* Cuatr. and *T. cacao* L. ssp. *sphaerocarpum* (Chevalier) Cuartr. corresponding to "Criollo" and "Forastero" (Cuatrecasas, 1964). According to later genetic studies this classification became obsolete (MOTAMAYOR et al., 2000) as it was proved that all cacao types are closer related than previously expected. Accordingly, MOTAMAYOR et al. (2008) identified nine genetic clusters of Forasteros in the Amazon basin named Amelonado, Contamana, Curaray, Guiana, Iquitos, Marañon, Nacional, Nanay, and Purús. Criollo was added as the 10th cluster. However, outside of science, this classification has as yet not been broadly applied.

Forastero type cacao originates from the upper Amazon region (Upper Amazon Forastero, UAF) and in the past also spread to the lower Amazon regions (Lower Amazon Forastero, LAF). Because of the characteristic fruit chape, both types are also referred to as "Amelonados" ("melon-shaped") (STOLL, 2010). Forastero type fruits are hardly furrowed, with some types completely smooth. Fruits are mainly green when unripe and change to yellow during ripening. Fruit shells are hard and usually consist of a double layer. One fruit contains between 30 to 60 large and flat seeds. Cotyledons are usually rich in anthocyanins which leads to a deep purple color that turns dark brown during fermentation. Forastero cacao trees are relatively resistant towards pest and diseases. Furthermore, yield can be high when farm management is adequate. However, Forastero cocoa beans usually do not contain fine and flavor aromas, but have a rather strong cocoa taste as well as bitter and astringent notes. Therefore, on the international cocoa market Forastero type raw cocoa beans are classified as "bulk cocoa" (LIEBEREI et al., 2010).

Criollo type cacao was bred by the indigenous human population in Middle America up to Mexico starting around 5.000 years ago. Due to the long separation from the original population in the upper Amazon region properties of Criollo cacaos became distinct. Today, Criollo type cacao is still cultivated in parts of Middle and South America. Criollo fruits have an oblong shape and a rough, deeply furrowed surface divided by longitudinal furrows and additionally possess a bend tip. The fruit color is mainly reddish both in unripe and in ripe state. Fruit shell is thin with a single layer. One fruit

usually contains between 20 to 40 small, oval seeds (LIEBEREI et al., 2010). As the indigenous population used it as a stimulating drug, Criollo cacaos contain up to 2 percent caffeine in the seeds (LIEBEREI AND REISDORFF, 2012). Cotyledons contain no or very few anthocyanins which is the reason why the seed color is white to creamy and turns light brown during fermentation. Criollo cacao varieties contain volatile compounds that are responsible for a fruity, flowery or nutty flavor of the seeds. In addition, they hardly have an astringent or bitter taste. Due to their aroma and flavor profile, Criollo raw cocoa beans are classified as "fine and flavor cocoa" which yields higher prices than Forastero raw cocoa beans. However, Criollo type cacaos are susceptible to pests and diseases and have a rather low crop yield in comparison to Forasteros (LIEBEREI et al., 2010; ROHSIUS, 2008).

A third cacao group, the hybrid between "Forastero" and "Criollo", is called "Trinitario". It is named after its first breeding place, the island Trinidad. Originally, Criollo type cacao was grown on Trinidad, but after the destruction of the majority of cacao plantations, Forastero cultivars from Brazil were introduced. Thereby, the two varieties hybridized which resulted in cacao showing the advantages of both former cacao types and an additional heterosis effect. Today Trinitario varieties are either classified as "bulk" or "fine and flavor" cacao, depending on the properties of each clone (ICCO, 2019).

"Nacional" or "Arriba" (the latter is the trade name) genetic group cultivars were planted in Ecuador starting from the early 1600s (BARTLEY, 2005). Originally, they derived from Upper Amazon Forasteros. Nacional raw cocoa beans are low in acidity, bitterness, and astringency, but possess fruity and floral flavor notes (BOZA et al., 2014). Therefore, Nacional cultivars are classified as "fine and flavor" cocoa and thus obtain higher market prices than bulk cocoa beans (LIEBEREI et al., 2010).

Within each genetic group, vegetatively propagated plant material is available with identical geno- and phenotypes, so-called clones. Many of these clones are kept in cacao gene banks all over the world with the largest in Trinidad at the University of the West Indies (LIEBEREI et al., 2010). The clones are given codes for identification that are usually based on their original growing locations or on the breeder and a consecutive numbering. All clones of international interest are registered and described in the International Cocoa Germplasm Database (ICGD). This study was performed with international Trinitario clones (ICS-1, ICS-6, ICS-39, ICS-60, ICS-95, TSH-565, UF-29 and UF-613) imported to Peru, with two Forastero clones (IMC-67 and CCN-51) as well as with undefined local cacao trees (ICS: Imperial College Selection, TSH: Trinitario Selected Hybrid, UF: United Fruit, IMC: Iquitos Mixed Calabacillo, CCN: Collección Castro Naranjal) (ICGD, 2020).

The role of CCN-51

The clone CCN-51 was developed by Homero Castro in Ecuador in the early 1960s from the following hybridizations: (ICS-95 x IMC-67) x Oriente-1. The latter clone was reported to be collected in the

Canelos Valley in the Ecuadorian Amazon (BOZA et al., 2014). Already in the 1960s, CCN-51 was taken to Peru and kept in gene banks, but not used in plantations until the 1990s (GÓMEZ ALIAGA et al., 2014). CCN-51 turned out to be pronouncedly resistant to different plant diseases and at the same time highly productive with yields around 2,000 kg ha^{-1} y^{-1} without shade, fertilizer or weed control. Therefore, it was planted in many areas in Ecuador, Peru and other countries. However, it is low in cocoa butter and also deficient in its flavor profile, with highly bitter, acidic, and astringent notes. For its flavor and further traits, it is classified as Forastero and therefore as bulk cocoa (ICGD, 2020). Consequently, CCN-51 was on the one hand a blessing, on the other a curse as it has high yields, but caused the decrease of the fine and flavor cocoa share in Ecuador, which caused a dropping in the ranking of the International cocoa organization (ICCO) from 100 to 75 percent and subsequently lower revenues for the farmers. Until today this dilemma is present (BOZA et al., 2014; ICCO, 2016).

1.2.3 Cacao distribution

In the past, it was unclear whether the cacao tree (*Theobroma cacao* L.) originates in Middle America in the area of Mexico or in the South American Amazonas region at the eastern slopes of the Andes (CLEMENT et al., 2010). Genetic analysis revealed the latter (MOTAMAYOR et al., 2002; ZHANG et al., 2006). However, the first cultivation of the cacao tree took place in Middle America mainly by the Maya people (LIEBEREI AND REISDORFF, 2012). In a first distribution phase, cacao was spread from the center of origin in the Amazon region of Ecuador, Brazil and Peru (Upper Amazon Forastero) downstream the Amazonas River up to the mouth (Lower Amazon Forastero). The second phase around 3000 BC involved the distribution from the center of origin to Venezuela and Middle America up to Mexico. There, the cacao tree was cultivated by the Olmecs, the Mayas, and by the Aztecs consecutively (LIEBEREI AND REISDORFF, 2012). Cacao beverages out of the pulp or the seeds were already consumed around 1100 BC which was discovered by theobromine traces in clay pottery (HENDERSON ET al., 2007). Cacao was selected and bred according to their preferences which was amongst others a high content of caffeine (approx. 2%), along with low levels of theobromine (BRUNETTO et al., 2007). Cocoa beans were also used for spiritual rituals and as a currency, partially until 1840. In contrast, the native nations in South America mainly used the fruit pulp of cacao. Therefore, cacao fruits in the Amazon region were not bred for aroma purposes of the seeds, for which reason they contain less caffeine (<0,2%), but are high in flavonoids (LIEBEREI AND REISDORFF, 2012).

Another cacao distribution phase took place around 600 years ago when Upper Amazon Forastero was distributed to coastal parts of Ecuador where it was cultivated and bred. Due to the separation from the original population quality traits evolved independently; today this variety is called “Cacao Nacional” (LIEBEREI ET AL., 2010).

In the 17th century, in post-Colombian times, Criollo cacao varieties were distributed to the Caribbean regions by the conquerors where they were cultivated (Fig. 2). Due to a natural disaster, the main part of the Criollo plantation was destroyed on the island of Trinidad around 1727. Therefore, cacao of the Forastero type was distributed from Brazil to Trinidad in order to replace the destroyed cacao populations. Criollo and Forastero varieties formed hybrids on the island and thus the "Trinitarios" with their high productivity, resistance, and fine and flavor aroma notes. Still today, hybrids between Criollo and Forastero type cacao are called "Trinitarios", irrespective of the location they are grown. As the consumption of cocoa and chocolate products increased also in Europe, the Spanish conquerors transferred Criollo type cacao from Middle America to other parts of the world around 1660 (Fig. 2). It was distributed across the Pacific Ocean to Malaysia, Indonesia, and other locations in the South Asian region. From there, further distribution took place to South India, Madagascar and around 1880 also to the region of Tanzania in eastern Africa. Trinitario cacao varieties were distributed from Trinidad to other parts of the Caribbean region as well as, in small amounts, to Western Africa and to Madagascar, South India, and the Malaysian and Indonesian regions, thus mixing there with the formerly transferred Criollo varieties (Fig. 2). In contrast, Forastero cultivars were distributed from Brazil to the Western African regions around 1822, these are also known as "West African Amelonados" (Fig. 2). This distribution pattern can coarsely still be found until today, despite various international breeding programs in different parts of the world (LIEBEREI et al., 2010; ROHSIUS, 2008; STOLL, 2010).

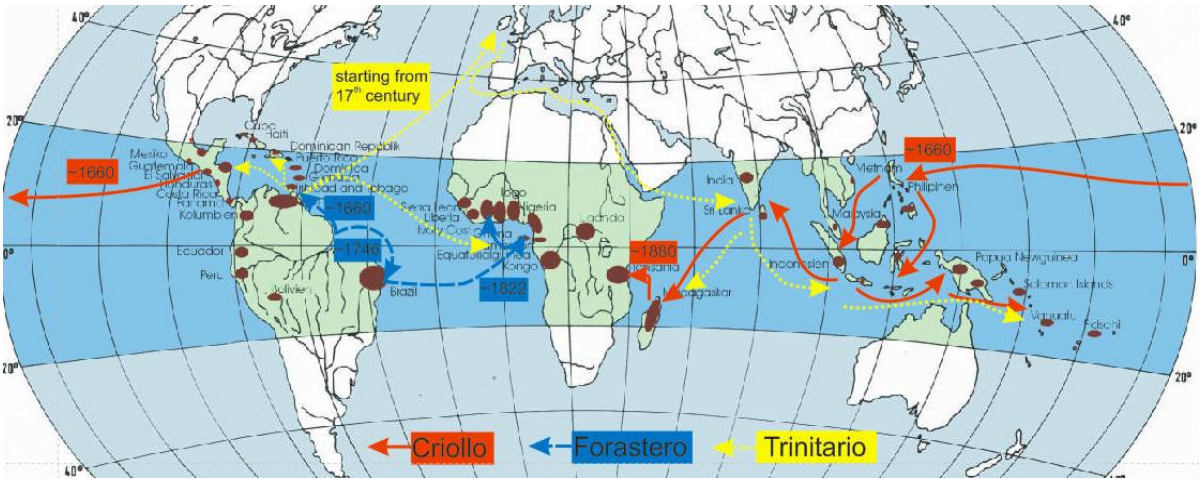

Figure 2: Distribution of *Theobroma cacao* L. from its primary growing and cultivation areas to other parts of the tropics. The three main cacao groups are displayed. Criollo cultivars: distribution from middle America to Asia, East Africa and Madagascar; Forastero cultivars: distribution from Brazil to West Africa and the Caribbean; Trinitario cultivars: distribution from Trinidad to the complete tropical belt and to the UK.

1.2.4 Cacao propagation and breeding

Breeding of cacao has different targets. It often aims at the improvement of yields by obtaining trees with higher fruit set as well as more resistant clones towards pest and diseases (PHILIPS-MORA et al.,

2005). Nowadays, as climate change affects areas of cacao cultivation, increasingly varieties resistant to drought and water logging are searched for (LAHIVE et al., 2019). Furthermore, lately aroma and flavor components of cacao are in focus as the demand of chocolates with high cocoa content has risen (BEKELE AND PHILIPS-MORA, 2019).

In the past, cacao was propagated by seedlings only and still is by many smallholder farmers, either with unknown parent combinations or with intentional crossings between certain clones (ICCO, 2000). However, the occurring segregation leads to a highly heterozygous population (MAXIMOVA et al., 2005) For vegetative propagation of cacao, three procedures can be applied: cuttings (using a part of a cut branch that develops roots), budding (using a bud put under a flap of bark of another tree) or marcotting (induction of root production at a branch by removing the bark) (ICCO, 2000). In Peru budding is mainly used (Fig. 3b). Propagation can be performed with either orthotrop or plagiotrop branches. However, each type of branch produces similar shoots. As trees should not grow high to facilitate harvesting, bushy forms are preferred. Therefore, fan branches (chupon parts) are joined as scions with the rootstock as lower part (FRANKE, 1994). Using vegetative propagation, genetic consistency is maintained by grafting a scion with desired fruit traits onto a rootstock. Additionally, using mature scions supports the ability to reduce the time span of first fertility from about 5 to 6 years to 2 to 3 years in cacao. In Peru, propagation of seedlings as rootstocks is usually done in cacao tree nurseries by cooperatives or the farmers themselves. Either the scions are grafted on the small rootstocks still in the tree nursery or the small rootstocks are transferred to the plantation area where grafting is caried out on site (own observation).

Another technique for the propagation of cacao is in-vitro-culture. Staminode material is used for the induction of somatic embryogenesis (MULLER, 2013). On lab scale, this method is well established for some cacao clones. Also, application on plantation level has been carried out. Advantages of this technique are the huge quantity of plantlets obtained as well as low costs. However, propagation protocols do not apply to every cacao variety yet. Furthermore, this technique has not yet reached smallholder farmers (MAXIMOVA, et al., 2005; MULLER, 2013).

Figure 3: **a** Young cacao trees propagated by seedlings in a tree nursery; **b** recently grafted cacao tree in plantation with cut rootstock and successfully inosculated scion, both in Peru.

1.2.5 Cacao cultivation and farm management

The cacao tree as a tropical understory tree is adapted to thrive in shady rain forests. Albeit in literature cultivation is possible up to 300 m of altitude (FRANKE, 1994), e.g., in the province of Huánuco in Peru, cacao plantations are located up to 800 m of altitude (own observation). Cacao is highly sensitive to droughts and cold weather conditions. Therefore, precipitation should be distributed evenly throughout the year with approx. 1500 to 2000 mm per year (LIEBEREI AND REISDORFF, 2012). Short dry periods of maximum three months can be tolerated by some cacao varieties. In addition, cacao requires high relative humidity, not less than 70 percent during the night. Average annual temperatures should be at least between 18 to 20 °C but not exceed 30 to 32 °C, without days under 15 resp. 10 °C. Temperatures lower than 15 °C can already damage the tree, whereas 10 °C is for sure harmful (FRANKE, 1994; ICCO, 2013).

Cacao trees needs proper soil properties for growing. Soils should contain coarse particles for aeration and water drainage as well as sufficient nutrients up to a depth of 1.5 m where the main part of the fine roots are located. The pH should optimally be in the range of 5.0 and 7.5. Hence, excessive acidity or alkalinity should be avoided. In case nutrient content of the soil is high, cacao is more tolerant towards acid soils. Organic matter content should be at approx. 3.5 percent within the top 15 centimeters. To be appropriate for cacao trees, soils need a certain anionic and cationic balance. To avoid nutritional problems, the exchangeable bases should amount to at least 35 percent of the total cation exchange capacity (CEC). The optimum total nitrogen to total phosphorus ratio should be around 1.5 (ICCO, 2013).

Cacao trees are cultivated in plantations of 1 to 5.000 hectares or more. Approximately 90 percent of cacao is produced by smallholders with less than 5 hectares of farmland (ICCO, 2012). Around 14 million people worldwide grow cacao, with 10.5 million in Africa (3.6 million in Ivory Coast, 3.2 million in Ghana), 1.39 million in America, and 2.11 million in Asia and Oceania (ICCO, 2012). In West Africa cacao produces on average 300 to 600 kg ha^{-1} y^{-1}, whereas in parts of South America and on large scale plantations cacao may produce up to 2,500 kg ha^{-1} y^{-1} (LIEBEREI et al., 2010).

Cacao is grown in different cultivation systems, in full-sun plantations or in agroforestry systems with either legume service shade trees (SOMARRIBA AND BEER, 2011) or shade trees as intercrops (ARMENGOT et al., 2016). Full-sun plantations function as cacao monocultures (see Fig. 4a) causing higher stress levels since cacao as a tropical understory tree is naturally adapted to shade (LIEBEREI AND REISDORFF, 2012). However, full-sun systems yield higher when fertilized and irrigated properly, but have a lower return on labor than agroforestry systems (ARMENGOT et al., 2016). Due to the stress level, life span of the cacao trees is shorter than in other cultivation systems. Because of the elevated maintenance costs, full-sun plantations are predominant on large scale farms. By the removal of shade trees,

smallholder farmers reduce their ability to adapt to global changes driven by food insecurity, cocoa price volatility, climate change, and demographic pressure (VAAST AND SOMARRIBA, 2014)

A classic example for a cacao agroforestry system exists in Bahia, Brazil, where the cacao trees are planted under the native trees of the thinned-out Atlantic Rainforest, called "Cabruca" (SAMBUICHI et al., 2012; Fig. 4b). In other crop shade tree systems banana resp. plantain shrubs (*Musa* sp.), Papaya (*Carica papaya* L.), maize (*Zea mays* L.), coconut (*Cocos nucifera* L.) or other palm trees are planted together with the cacao trees (BENTLEY et al., 2004; Fig. 4c). Especially banana or plantain shrubs are planted in a first step to provide the subsequently planted young cacao trees with shade. After about three years, the banana shrubs are taken away leaving space for the growing cacao trees. During these years, the bananas or plantains provide an income for the farmer (GÓMEZ ALIAGA et al., 2014). Furthermore, timber trees are often planted around and within the plantation as shade trees. Worldwide, *Gliricidia sepium* (Jacq.) Steud. is a preferred shade tree in cacao plantations. In Middle America, this species even has the common name "madre de cacao" ("mother of cacao"). Belonging to the Fabaceae family, it provides nitrogen fertilization to soils by its symbiotic relationship with rhizobial bacteria (KABA et al., 2019). Also, *Inga edulis* Mart. is in South America frequently used as shade tree, mainly because it has a symbiotic relationship with the rhizobial bacteria strain *Bradyrhizobium* (LEBLANC et al., 2005). Usually, timber trees are harvested at about 20 years of age and hence at the same time when cacao trees have to undergo a rejuvenation, which provides an additional income to the farmers. Intercropping of cacao trees is also done with rubber trees (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.), different palm trees (mainly oil palm *Elaeis guineensis* Jacq. and coconut *Cocos nucifera* L.), citrus trees and others (KOKO et al., 2013).

Spacing of cacao trees depends on climate and soil conditions, cacao varieties, and cultivations systems (FRANKE, 1994). However, it was shown that agroforestry systems do not increase pest and disease incidence compared with monocultures when managed adequately (ARMENGOT et al., 2020). Common spacings are 3 m by 3 m in a square or in triangles respectively in rectangles, which results in 1,100 to 1,500 cacao trees per hectare (GÓMEZ ALIAGA et al., 2014). However, a denser spacing does not necessarily lead to higher yields as the root system and tree crown need sufficient space for growing. Crown and root diameter range between 2.5 to 6 meters (STOLL, 2010). Furthermore, agroforestry systems play a pivotal role to support biodiversity conservation (ASARE, 2006).

Cacao agroforestry and intercropping systems have been under study for a long time in different regions of the world. However, the underlying mechanisms of competitive and complementary effects are so far poorly understood. Especially the complex interplay of shade/intercropped trees and naturally growing shrubs and herbs with the cacao trees have hardly been studied (ZUG et al.,

2019). Furthermore, the effect of the abundance of species and their evenness in relation to cacao quality traits as well as yield have so far not been investigated.

Figure 4: Cacao plantations with **a** monoculture of newly planted cacao in a full-sun system in Peru; **b** cacao system "Cabruca" in Bahia, Brazil with added banana shrubs; **c** young cacao trees intercropped with banana and coconut in Peru.

Cultural management practices are a crucial factor for stable and high yields (SOBERANIS et al., 1999). Cacao trees undergo a form pruning after the first year of growth where the tree is cut to the desired growth form. Often the main stem is kept short dividing into three main, equal branches as trunks (GÓMEZ ALIAGA et al., 2014; FEDERACIÓN NACIONAL DE CACAOTEROS, 2015). Usually once a year, a maintenance pruning is done where the tree is cut into a wineglass shape with a height of about 3 to 5 meters (LIEBEREI et al., 2010; Fig. 5 b,c). Some farmers cut the tree differently to obtain a double treetop for self-shading. The latter technique is mainly applied in full-sun plantations without shade trees (pers. comm. REINHARD LIEBEREI). Furthermore, phytosanitary pruning is applied whenever necessary (GÓMEZ ALIAGA et al., 2014; FEDERACIÓN NACIONAL DE CACAOTEROS, 2015; Fig. 5a). Cultural management practices also include fertilization and crop protection by pesticides, if necessary (GÓMEZ ALIAGA et al., 2014; FEDERACIÓN NACIONAL DE CACAOTEROS, 2015). Depending on the farm organization this is either done with conventional or organic products (ARMENGOT et al., 2016; Fig. 5b). In cacao, apart from soil fertilization also leaf fertilization is common to apply macro- and micronutrients (GÓMEZ ALIAGA et al., 2014; Fig. 5c).

Although it seems obvious that farm management plays an important role for cacao yield, the further effects of pruning, fertilization and pest management have scarcely been studied systematically. Especially the influence of conventional fertilization and pest management versus organic farming has hardly been analysed (ARMENGOT et al., 2016). Due to the increasing relevance of cocoa bean quality, also the influence of farm management on quality traits should be further investigated.

Figure 5: Farm management on cacao farms in Peru. **a** Phytosanitary pruning; **b** organic soil fertilization; **c** organic leaf fertilization; **b** and **c** pruned cacao tree with wineglass shape and three main stems.

1.2.6 Cacao pests and diseases

Depending on the variety, cacao is more or less susceptible to pests and diseases that occur at varying frequency in different parts of the world. In Asia and Oceania, a moth called "cocoa pod borer" (*Conopomorpha cramerella* (Snellen)) causes severe yield losses by infesting pods (BRADLEY, 1986). In West-Africa the tree itself can get infected by the so called "cacao-swollen-shoot-virus", that relates to seven species of the genus *Badnavirus* within the *Caulimoviridae* family. The virus is transmitted by mealybugs and infects exclusively the cacao tree. As the trees die from the easily transferred infection, it can result in a severe economic loss for the cacao farmer (JAQUOT et al., 1999).

In South America, mainly the fungal diseases "witches' broom" (*Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora), "black pod rot" (*Phytophthora* spp. de Bary) and "frosty pod rot" (*Moniliophthora roreri* (Cif.) H.C. Evans, Stalpers, Samson & Benny) (see Fig. 6) cause severe cacao yield or even entire farm losses (FULTON, 1989). Witches' broom disease is currently present in South America, Panama, and the Caribbean caused by an agaricomycete infecting the stem, flower cushions and fruits. The name originates from the notable symptoms of the infected cacao trees showing abnormal broom-like structures of the shoots. *Moniliophtora perniciosa* is native to the Amazon region and was transferred to Bahia, Brazil, in 1989 resulting in a disastrous infection scenario reducing the production of cacao in that area by more than 75 percent (GRAMACHO et al., 2016). Still today, cacao farming in Bahia has not fully recovered, which explains the ongoing search for resistant cacao clones (LOPES et al., 2011).

Also in Peru, witches' broom is present in many cacao farms causing yield loss (Fig. 6a, b). Black pod rot is caused by a genus of oomycetes that infect thousands of plant species worldwide. In cacao it is also present but not as destructive as the other two fungi mentioned (Fig. 6c).

Figure 6: Main cacao diseases in South America, all caused by fungi: **a** and **b** **"**witches' broom" (*Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora) with **a** infestation of branches; **b** infestation of pod; **c** "black pod rot" (*Phytophthora* spp. de Bary); **d** "frosty pod rot" (*Moniliophthora roreri* (Cif.) H.C. Evans, Stalpers, Samson & Benny).

Frosty pod rot is a basidiomycete fungus that is one of the most serious problems for cacao production in Latin America as it can lead to up to 90 percent yield losses and total field abandonment. Its name "frosty pod" is related to the appearance of powdery conidia on the infected mature pod with more than seven billion spores (BAILEY et al., 2018; Fig. 6d). So far *Moniliophthora roreri* has spread to Middle America up to Mexico as well as to the northern and western parts of South America, but not yet to Brazil (PHILIPS-MORA et al., 2007). Phytosanitary treatments are applied to reduce the fungus (KRAUSS et al., 2003) but also research on resistant cacaos is carried out (PHILIPS-MORA et al., 2005). It is clear that all fungi prefer warm, humid climate for propagation. However, the complex interplay of cultural management techniques and species composition with the incidence of the fungi have rarely been studied (ARMENGOT et al., 2020; BOS et al., 2007). Furthermore, it remains unknown whether cacao infestations influence cocoa bean quality traits.

1.3 Cocoa production and use

1.3.1 Postharvest treatments – Fermentation and drying

Ripe cacao pods remain at the tree and do not fall off. It is important to harvest the fruits in the right ripening stage as unripe pods do not yet contain the desired storage proteins, fats, and sugars for later aroma formation. These compounds develop only within the last three weeks before maturation, with protein degrading enzymes being synthesized even later. In contrast, overripe fruits should also be avoided as pulp dries out and seeds may begin to germinate within the fruit, both leading to changed fermentation conditions (LIEBEREI ET AL., 2010). However, farmers tend to pick the fruits as early as possible for economic reasons. Fruit maturation is difficult to be recognized in some varieties as hardly any color or shape changes are notable. Ripe fruits are harvested with care usually using a machete in order to avoid the destruction of the sensitive flower cushions. Pods are then opened and the seeds with the fruit pulp collected in buckets or other transportation vessels. For

facilitation of the fermentation process, contaminations with pod husk residues, cacao placenta, infested beans etc. should be avoided (BCCCA, 1996; SCHWAN AND WHEALS, 2004). The empty husks usually remain in the plantation and can be used as natural fertilizer.

Fermentation aims at the removal of fruit pulp, reduction of the astringent and bitter substances inherent to the fresh seeds, and most of all the formation of precursors of the characteristic cocoa and chocolate aroma (LIEBEREI ET AL., 2010). These formations continue in the subsequent drying process.

It is common to change nomenclature in accordance with the processing steps. Therefore, in this study the term “cacao” is used for the description of the cacao tree, field, pod and related topics as well as for cacao seeds as long as they are capable to germinate. After fermentation and drying, the term “cocoa" is used. In addition, the term “seed” changes to “bean”. Fermented and dried beans are called “raw cocoa beans”, when roasted “cocoa beans”. Subsequent cocoa derivates are called cocoa powder, cocoa butter, and cocoa liquor (ITC, 2001; SETHI, 2018). In order to distinguish fermented and dried beans from pure dried beans the term “dried cacao seeds” is used in this study although they are no longer capable to germinate.

Apart from the genetic background (NIEMENAK et al., 2006; KONGOR et al., 2016) and the farming conditions (e.g., soil conditions, shade management; see ELWERS et al., 2009 and KIECK et al., 2016), also the process of the post-harvest treatments — fermentation and drying — influence the quality of raw cocoa beans (e.g., APROTOSOAIE et al., 2016; KONGOR et al., 2016). Depending on the cacao growing region and the cacao variety, fermentation and drying procedures vary. On the one hand fermentation techniques are often monetarily limited so that sophisticated fermentation vessels and drying stations are not available. On the other hand, diverging fermentation traditions and local adaptations have developed in different parts of the world. In Africa, fermentation is often conducted by using banana or plantain leaves lying on the ground on which the seed mass is piled up and then wrapped in. The banana leaves function as insulation and also as natural inoculation as they contain yeasts which promote the fermentation process. In other parts of the world, cacao fermentation takes place in bags, baskets or boxes, mostly also using in addition banana or plantain leaves (SCHWAN AND WHEALS, 2004). In Peru, the most common way are square wooden fermentation boxes with holes at the bottom for pulp drainage (Fig. 7). Such boxes may vary in wood type and dimensions and are made for cacao seed masses between 200 and 2,000 kg. For fine and flavor cacao, usually moderate quantities are fermented. However, amounts of less than 100 kg of wet beans are not recommended for normal fermentations. Also, fermentation with a mass larger than 2,000 kg may be non-effective or non-uniform (BCCCA, 1996). For natural inoculation, the fermentation mass is left open at the beginning of fermentation to enable the entering of fruit flies

that carry microorganisms at their tarsi into the fermentation mass. Also, unclean boxes and handling material along with the skin contact of workers are useful as inoculation (SCHWAN AND WHEALS, 2004). Furthermore, SCHWAN AND WHEALS (2004) recommend to use defined starter cultures to promote a well-ordered succession of microorganisms. However, in Peru experiences with starter cultures are so far lacking.

The interval between harvesting and opening the pods to start fermentation makes a difference for the fermentation process and the rise in fermentation temperatures. The extent of flavor improvement by storing the pods prior to fermentation varies considerably between genotypes (BCCCA, 1996). Both genotype and climate conditions (e.g., precipitation) influence the volume and sugar content of the fruit pulp as well as the composition and intensity of the aroma compounds of the pulp (HEGMANN, 2015). According to MOTAMAYOR et al. (2000), pulp of Criollo cacao cultivars have a higher sugar content than Forastero varieties. Some cacao types, especially in Malaysia, but also CCN-51 of Ecuador, have more pulp than other cacao types, as indicated by lower seed to pulp ratio (BIEHL et al., 1989). This pulp abundance leads to extremely acidic raw cocoa beans after the fermentation and drying process, which finally translates to poor chocolate quality. In Brazil, pulp removal of up to 20 percent proved to reduce this problem without consequences for the fermentation process. In Malaysian raw cocoa beans, the treatment did not lead to reduced acidity, only a pre-drying of the seeds showed positive results. This may be attributed to the fact that cacao cultivars in Malaysia, have approx. three times more pulp sugars than Brazilian “comum” cultivars (SCHWAN AND WHEALS, 2004). Besides the improved fermentation results after pulp pre-treatments, the drained cacao pulp can also be used as an additional income source by producing jellies, jams, vinegar and liquor. Such commercial products are already produced or under development in different cacao-producing countries, such as Brazil, Malaysia and Ghana (ODDOYE et al., 2013). In Peru, a protocol for an adequate fermentation process along with possible pulp pre-conditioning techniques is still missing for the recently introduced Trinitario cultivars. In particular, the quantities of pulp need to be monitored clone-wise to establish adapted fermentation procedures. Commercial products of the cacao pulp are so far not found on the Peruvian market.

Apart from pulp, also bean size is an important parameter in fermentation. Beans should be of homogeneous size to avoid over- or under-fermentation. Currently there is no internationally accepted bean size classification, but some origins and markets defined their own criteria. Bean size is controlled after fermentation and drying through the so-called “100-bean-count” or “100-bean-weight”. Thereby 100 beans have to weigh more than 100 g or beans are counted to obtain the number of beans per 100 g (BCCCA, 1996). The main influence factor of bean size seems to be the rainfall regime during pod development. As rainfall during the first 2 to 3 month of pod growth is

correlated with mean bean weight, pods developing in the wet season tend to have larger beans than those developed during the dry season (BCCCA, 1996).

Fermentation starts with a microbial transformation of the pulp which is characterized by high contents of pectin, citric acid (pulp pH 3.6) and sugars (14%, with 60% sucrose and 39% a mixture of glucose and fructose). Various different yeast species are present in fermentations with variations depending on the geographical location and fermentation method (SCHWAN AND WHEALS, 2004). The yeasts transform the pulp sugars to ethanol and carbon dioxide (STOLL, 2010). This anaerobic yeast phase lasts approximately the first 24 to 36 hours of fermentation (APROTOSOAIE et al., 2016). Heterofermentative lactic acid bacteria decompose pulp citric acid (KRÄMER AND PRANGE, 2017) which leads to a rise of the pH value of the fermentation mass. In consequence temperature increases to approx. 28 to 35 °C. These conditions are optimal for microaerobic lactic acid bacteria that transform the glucose to lactic acid. Different species of lactic acid bacteria are present during fermentation, depending on fermentation duration, geographical location or differences in the process. This phase lasts approximately from 24 to 72 hours (LIEBEREI et al., 2010).

In addition, some yeast species secrete pectinolytic enzymes which together with plant-owned enzymes degrade the pulp (APROTOSOAIE et al., 2016; SCHWAN AND WHEALS, 2004). As a result, the pulp is liquified and drains off. This process is usually completed 24 to 36 hours after fermentation start (SCHWAN AND WHEALS, 2004; STOLL, 2010). Due to pulp drainage, oxygen enters through gaps into the fermentation mass. To promote additional aeration, the fermentation mass is turned (BCCCA, 1996), but the turning method is handled differently in the cacao growing regions and with different cacao types. In some cases, no or maximum one turning per day is performed, in others turning is done twice or three-times during fermentation. Turning may also be carried out within the same box or by transferring the fermentation mass from one box to another (LIEBEREI et al., 2010). Because of the incoming air, an aerobic phase starts in which the pulp ethanol is oxidated to acetic acid by acetic acid bacteria. The acidification (pH 4 to 4,5) is accompanied by a steep temperature increase to between 45 and 50 °C caused by the exothermic reactions catalyzed by acetic acid bacteria. Furthermore, organic acids start penetrating the seed's testa leading to the embryo's death and the subsequent destruction of cell compartmentation (BIEHL, 1973). As the testa functions as a barrier, first entrance of acids occurs in the area of the micropyle. With the progression of fermentation, higher temperatures, and more acid formation, the seed shell seems to become more permeable (ROHSIUS, 2008). Subsequently, proteolytic cleavage of storage proteins and sugars take place. Furthermore, polyphenols are oxidized as the PPO (polyphenol oxidases) that were formerly stored in idioblasts get in contact with the polyphenols, which is visible as a browning of the seeds. The last part of fermentation lasts approximately from 48 to 112 hours. At the end of fermentation, pH level of the seeds rises to approx. 5 to 5.3 (STOLL, 2010).

As described, microorganisms are essential for a successful course of cacao fermentation. Species of yeasts, lactic and acetic acid bacteria need to be present whereas microbial diversity differs with location and process parameters (DANIEL et al., 2009; KOSTINEK et al., 2008). Some microbial strains were identified to be present more often than others, these include the yeasts *Hanseniaspora guilliermondii*, *Pichia kudriavzevii* and *Kluyveromyces marxianus, the lactic acid bacteria Lactobacillus plantarum*, *Lactobacillus fermentum* as well as the acetic acid bacteria *Acetobacter pasteurianus* and *Gluconobacter frateurii* (APROTOSOAIE et al., 2016). According to SCHWAN AND WHEALS (2004) members of the genus *Acetobacter* occur more often than *Gluconobacter*.

Spore forming bacteria and filamentous fungi seem to be present throughout the entire fermentation, but in small quantities. At the end of fermentation when temperature drops and acetic acid bacteria become less present, those bacteria and fungi may proliferate and produce organic acids formation and off-flavors. For instance, SCHWAN AND WHEALS (2004) suggested that free fatty acids causing off-flavors are produced by different species of the genus *Bacillus*.

In general, cacao seeds of cacao varieties classified as bulk cocoa are fermented for up to 8 days. In contrast cacao seeds of cacao varieties classified as fine and flavor are fermented for a shorter period, with some Criollo types taking 2 to 3 days only (AFOAKWA et al., 2008; SALTINI et al., 2013). Fine and flavor cocoa fermentation aims at both producing the typical chocolate flavor through proteolytic degradation of storage proteins and at conserving the clone-specific aroma components (LIEBEREI et al., 2010). Terpenoids like linalool causing floral aroma notes as well as esters with organic acids causing fruity aroma notes are found in the maternal pulp tissue. While the latter are fat soluble and thus remain within the seed after entrance, terpenoids are not acid-stable which is the reason why a prolonged fermentation process reduces their presence (KADOW et al., 2013). However, fermentation procedures for fine and flavor cacao clones from South America have not yet been studied comprehensively.

After fermentation, a slow but continuous drying process should follow that aims at reducing the water content in the beans to between 6 to 8 percent as well as the acid content within the beans (LIEBEREI et al., 2010). Water content should be low to prevent the development of molds during transport and storage. As acetic acid is volatile, it can evaporate from the beans during drying, but it can also be reduced later during chocolate production, whereas lactic acid being non-volatile remains in the raw cocoa beans once it is formed. If lactic acid is present in excess, it can cause off-flavors in chocolate. In general, acids at high quantities may be an indication for a deficient formation of aroma precursors. Therefore, dry beans should have a pH above 5.0 (BCCCA, 1996). Furthermore, the drying process should be slow to permit evaporation of water and acetic acid. Fast drying may result in an encrustation of the inner part of the testa which then functions as a barrier and impedes that surplus

acetic acid leaves the seed (ROHSIUS, 2008). In contrast, prolonged drying may lead to the growth of molds. Therefore, periodic turning of the beans and a thin bean layer during drying are a relevant measure. As long as the beans have an elevated water content the biochemical changes of the fermentation continue. Drying lasts between 6 to 14 days and can be done by open sun drying or artificially. Sun drying is found to lead to better results than artificial dryers as the latter often dry at too high temperatures (WOOD AND LASS, 1989). Drying of cocoa beans is often done at inadequate places, e.g., alongside the road on plastic tarpaulins or where animals (often chicken) are present. Sun drying should take place at dry and clean places, preferably elevated from the ground on wooden tables or on nets where animals cannot enter (LIEBEREI et al., 2010; Fig. 7). In addition, beans should be protected against rainfall and moisture. Especially during the rainy season, drying of raw cocoa beans can be challenging and artificial drying may be a better alternative. Of course, smoke or other contaminants should be avoided during drying (STOLL, 2010).

During fermentation and drying, aroma precursors are produced through biochemical reactions. Seed globulins (storage proteins) are hydrolytically cleaved to hydrophobic oligo-peptides at a low pH-level of optimally 3.5 by aspartic endoprotease. In a next step, these compounds are decomposed to hydrophilic oligo-peptides and hydrophobic amino acids by carboxypeptidase activity at a pH optimum of 5.8. The carboxypeptidase needs proteolytically formed peptides as a substrate as it does not cleave proteins (LIEBEREI et al., 2010; STOLL, 2010). The resulting compounds are the essential aroma precursors for the formation of the typical chocolate aroma that ultimately develops in the Maillard reaction during roasting (e. g., VOIGT et al., 1994; GIACOMETTI et al., 2015). At the same time, bitterness and astringency are reduced by polyphenol degradation (OLIVIERO et al., 2009; KONGOR et al., 2016). Fermentation methods, duration, mixing intervals and different treatments are adjusted based on the cacao variety and the environmental conditions (e.g., SALTINI et al., 2013).

During the fermentation process and afterwards, determination of the fermentation degree is used as a quality criteron. Therefore a cut test of the beans is performed where the beans are cut lengthways through the middle either with a knife or with a special raw cocoa guillotine (50 beans are cut simultaniously). Well fermented beans have a brownish color (in Forasteros darker than in Criollos) as well as a furrowed structure. Unfermented beans possess a slaty color and an unstructured, waxy consistency. Underfermented beans show a violett color and and intermediate structure. Furthermore, insect infestation of the beans, germinated beans (noteble by a hole at the radicle part of the bean), clustered beans, broken or cut as well as flat beans are also undesired and result in a lower quality grade (BCCCA, 1996). Beans that are not well fermented or have one of the stated defects do not lead to the desired aroma and flavor profile or even cause so called “off-flavors”.

Figure 7: Fermentation and drying facilities in Peru with **a** box stair fermentation facility; **b** open solar drying on tarpaulins; **c** solar drying on wooden tray trolleys with rain protection.

After drying, raw cocoa beans should be used locally or transported to the country of consumption as soon as possible. Long storage periods in tropical countries should be avoided to prevent re-humidification that may result in mold growth and insect infestation. Usually, transport takes place by ocean vessels in bulk containers or in bags. Also, during transport, humidity has to be kept low (STOLL, 2010). Usually, containers are lined out with cardboard material or other materials with similar properties, which may cause contamination of the beans with mineral oils (MOSH: Mineral Oil Saturated Hydrocarbons, MOAH: Mineral Oil Aromatic Hydrocarbons) (DINGEL AND MATISSEK, 2018). Also, the frequently used jute bags may be a contamination source of mineral oil. Therefore, CAOBISCO (Association of Chocolate, Biscuits and Confectionery Industries of Europe) has launched a specification for food grade bags (BCCCA, 1996).

1.3.2 Cacao and cocoa composition and flavor

Depending on the cacao genotype, aroma and flavor components vary. Flavor thereby means the whole organoleptic impression including gustatory, olfactory, and tactual aspects, whereas taste refers to the sense inside the mouth and aroma to the sense of smell (ZIEGLEDER AND BIEHL, 1988). Aroma compounds are chemical compounds that possess a smell or an odor. Also, climate and soil conditions as well as farm management influences the aroma and flavor profiles. However, so far there are only few studies about these correlations (e.g., SALTINI et al., 2013). Cacao seeds that do not undergo fermentation but only a drying process do not develop cocoa flavor during roasting. Hence, fermentation and subsequent drying are required for the formation of cocoa flavor precursors (SCHWAN AND WHEALS, 2004). These cocoa or chocolate aroma derives from seed endogenous components (storage proteins and carbohydrates) (KADOW et al., 2013). Phenolic substances and alkaloids are inherent to the seed already before fermentation and are reduced in the course of the process (BRUNETTO et al., 2007). Other aroma relevant components that are characteristic for fine and flavor cocoa cultivars are located in the fruit pulp (e.g., monoterpenes, esters, methylketones). These merge into the seed during fermentation when testa becomes permeable (KADOW et al, 2013).

Fermented, dried and deshelled raw cocoa beans contain approximately 50 to 60% cocoa butter (fat), 11.5 to 14% protein, 9% cellulose, 7.5% starch and pentosans, 5 to 6% water, 2.6% minerals, 2% organic acids, 1.5% theobromine, 1% sugars and 0.3% caffeine (TERNES et al., 2005). These values vary depending on the cacao type, climate and soil conditions, and farm management.

Storage fats (cocoa butter) are located in lipid vacuoles of the cacao seeds. Forastero cacaos usually have a higher amount of cocoa butter (55-60%), whereas Criollo and Trinitario cultivars have lower contents (approx. 53%) (WOOD AND LASS, 1989). The main constituent of cocoa butter are triglycerides derived from palmitic acid, stearic acid, and oleic acid. Cocoa butter is high in saturated fats with 57-64% of the total fat content. The proportions of the fatty acids again differ depending on the genotype and growing conditions. Low temperatures during the last phase of fruit ripening leads to a higher content of unsaturated fatty acids, which results in softer butter (ROHSIUS, 2008). Such differences in the physical properties of the cocoa butter also affect the chocolate manufacturing process and recipes, with harder cocoa butter being preferred. Cocoa butter from Southeast Asia is the hardest, the one from West Africa intermediate, and butter from Cameroon and Brazil the softest. Peruvian cocoa butter varies between soft and intermediate depending on genotype and cultivation altitude (CHAISERI AND DIMICK, 1989). Due to the high amount of fat in the cacao seeds and beans, they are particularly susceptible to off-flavors that stay in the fat phase, e.g., smoky off-flavors caused by smokes or hammy off-flavors caused by over-fermentation (BCCCA, 1996).

A further quality criterion for cocoa butter is the content of free fatty acids (ffa). The higher the content in ffa the poorer the quality. Content should be less than 1 percent. Contents above 1.75 percent exceed the legal limit for cocoa butter in the EU. Elevated ffa levels are caused by broken beans, infested pods in the fermentation mass or by the formation of molds during drying, storage or transport (BCCCA, 1996).

Storage proteins are located in aleurone vacuoles of the mesophyll cells (BIEHL, 1973). 90 percent of the proteins in ripe cacao seeds are made up of albumins and globulins (VOIGT et al., 1994), with albumin making up 25-30 percent of the total proteins. Also, protein contents and compositions vary according to genotypes (ROHSIUS, 2008). During fermentation, the content of free amino acids rises due to the enzymatic cleavage of the storage proteins by the respective proteases. Not all amino acids form at the same time during fermentation, depending on the respective proteases. Most free acidic amino acids are metabolized during fermentation, whereas leucine, alanine, phenylalanine and tyrosine are considerably accumulated, most probably due to the specific activity of endopeptidases (KIRCHHOFF et al., 1989a). Amino acids and oligopeptides are along with reducing sugars (from sucrose, glucose, fructose and glycosides) responsible for hundreds of volatile compounds present in roasted beans. They are therefore indispensable for the desired cocoa and chocolate aromas

(SCHWAN AND WHEALS, 2004). Hence, examining the formation of free amino acids before, during and after fermentation reveals valuable insights into the fermentation process.

Like in most other plants, also polyphenols are present in cacao. Biologically many polyphenols like flavan-3-ols and procyanidins are supposed to function as protection against herbivores and fungi. In case of plant tissue damage, they are liberated and condensed with proteins in a reaction catalysed by polyphenol oxidases, resulting in insoluble complexes which heavily reduces the protein availability of the damaged tissue (STOLL, 2010).

In cacao three groups of polyphenols, that all belong to flavonoids, can be distinguished: anthocyanins (approx. 4%), catechins or flavan-3-ols (approx. 37%), and proanthocyanidins (approx. 58%) (BELITZ et al., 2007). All these phenolic substances belong to the flavonoid group that possess two aromatic rings (C_6-C_3-C_6) (LIEBEREI AND REISDORFF, 2012). The polyphenol storage cells (idioblasts) represent 10 to 12 percent of the mesophyll of the cotyledons (BIEHL, 1973).

Approximately 4 percent of the total polyphenol content are glycolyzed anthocyanins (BELITZ et al., 2007), mainly cyanindin-3-galactoside and cyanidin-3-arabinoside. They are present in cacao seeds of Forastero and partly in Trinitario cultivars. As they absorb visible light, the cacao seeds appear pink to dark violet (STOLL, 2010). During fermentation anthocyanin content has been reported to decrease due to conversion to complex tannins and anthocyanidins (WOLLGAST AND ANKLAM, 2000).

The main other important group of polyphenols found in cacao seeds are monomeric flavan-3-ols. With approx. 35 percent of the total polyphenol content (−)-epicatechin is the most important one, but also its diastereomeric counterpart the (+)-catechin is present. In cacao seeds, these compounds occur also as oligomers, the procyanidins (STOLL, 2010). In general, there seem to be no differences in total polyphenol and (−)-epicatechin contents between genotypes. However, soil fertilization may lead to significantly smaller amounts of total polyphenols, flavan-3-ols and anthocyanins within the cacao seeds. During fermentation and drying, catechins diminish more in Criollos than in the other genotypes which may be the reason for the mild flavor of chocolate made from these beans (ELWERS et al., 2009). Despite these findings, detailed research on factors influencing the content of polyphenols in cacao seeds is still missing.

In some cases, polyphenols, especially the antioxidant effect of (−)-epicatechin, have shown to have beneficial effects on health (RUSCONI AND CONTI, 2010). However, polyphenols are also partly responsible for the bitter and astringent flavors of the cacao seeds. During fermentation, a part of the polyphenols diffuses out of the beans or is oxidized by the polyphenol oxidase to mostly insoluble tannins (SCHWAN AND WHEALS, 2004). Due to the increasing interest in the healthy traits of cocoa derivates, efforts are being made to on the one hand avoid undesired tastes and on the other hand maintain as much polyphenols as possible (SCHWAN AND WHEALS, 2004).

The stimulating effect of cacao is based on theobromine, an alkaloid closely related to caffeine, as well as on caffeine itself (LIEBEREI AND REISDORFF, 2012). Again, both compounds are also partly responsible for the bitter taste of seeds and beans (SCHWAN AND WHEALS, 2004). The methylxanthines theobromine and caffeine are distributed in the cacao seed, pulp, testa, and accordingly after fermentation in the bean. In unfermented cacao seeds, the methylxanthines are stored in polyphenolic storage cells (STOLL, 2010). In raw cocoa beans, the content of theobromine is approximately twice as high as the content in caffeine. The third methylxanthine, theophylline, can be detected in traces only. During fermentation, a first increase of methylxanthines can be observed, most probably caused by the migration from the pulp though the permeable seed shell into the bean. Migration is here considerably higher in Criollo than in Forastero varieties (BRUNETTO et al., 2007). After the first increase, however, a decrease can be observed in the course of fermentation. Contents of methylxanthines vary considerably depending on the genotype with Criollo cultivars having low contents in theobromine but high ones in caffeine, whereas in Forastero varieties the situation is vice versa. Therefore, the relation between theobromine and caffeine content can be used as classification of the genetic variety. Trinitario cultivars as hybrids possess intermediate levels of methylxanthines (BRUNETTO et al., 2007). Despite the knowledge on cacao varieties, it remains unclear whether also environmental factors and farm management influence the methylxanthine levels in cacao.

The most commonly known flavor attributes of fermented and roasted cocoa beans are cocoa and chocolate notes (LIEBEREI et al., 2010). These are predominantly found in Forastero cocoas and are therefore characteristic for bulk cocoa. In contrast, fine and flavor cocoas have a less pronounced cocoa aroma. Depending on the genotype as well as on regional differences like climate, soils, and farm management their flavor attributes vary. Criollo cultivars from Venezuela possess a nutty, floral flavor profile, whereas Nacional from Ecuador has floral, fruity along with raw/bean/green attributes. Trinitario cultivars from Trinidad have an acid and fruity flavor profile (LIEBEREI et al., 2010).

Planting the same cacao genotypes in different regions of the world results in a different flavor profile. Therefore, for the lately introduced and cultivated Trinitario cultivars in San Martín, Peru, experience is lacking for optimal cultivation and post-harvesting treatments in order to obtain favorable flavor notes.

1.3.3 Roasting

30 volatile compounds and more than 400 other flavor components were identified in cocoa beans (LIEBEREI et al., 2010). For the formation of cocoa and chocolate aroma, roasting of the dry raw cocoa beans is essential. The aroma precursors such as free amino acids and oligopeptides that result from fermentation along with reducing sugars undergo a nonenzymatic browning through Maillard

reaction accompanied by the formation of various reaction products. Additionally, carbonyl compounds resulting from lipid oxidation can be detected (OLIVIERO et al., 2009). Maillard reaction together with oxidation, condensation, and complexation of polyphenol compounds and subsequent protein and starch hydrolysis are responsible for the formation of the characteristic brown color, pleasant aroma, and texture of roasted beans (OLIVIERO et al., 2009). Roasted bean properties, such as the concentration of volatile flavor compounds, total acidity and fat content depend on roasting conditions, mainly temperature and time of the process. Furthermore, the quality of the final product seems to result also from the thermal processing of cocoa beans, such as humidity and air flow rate (KRYSIAK, 2005). Roasting usually lasts between 10 and 45 minutes at temperatures between 70 to 140 °C. Roasting time and temperature are adapted according to the cacao genotype with Criollo cultivars having shortest times and lowest temperatures to maintain volatile compounds. Moreover, it depends on process type, roasting equipment, and consumer's preferences. Most commonly whole bean roasting is applied, which leads to detaching of the cocoa testa from the cotyledons which then can be separated in a subsequent process of deshelling. The resulting cocoa nibs are ground to cocoa liquor (pers. comm. NORMANN WAGNER). This intermediate product can be used for the production of different final products (STOLL, 2010).

1.3.4 Utilization of cocoa

From the cocoa liquor, cocoa butter and cocoa powder can be obtained by a pressing process. During the procedure with high pressures, cocoa butter and powder are separated. It is used for different applications, e.g., as a chocolate ingredient, in the food industry, in pharmaceuticals, cosmetics and perfumes (LIEBEREI AND REISDORFF, 2012).

Cocoa powder remains as a press cake after the extraction of cocoa butter. Cocoa powder can either be strongly de-oiled with a residual cocoa butter content of 11-12 percent or slightly de-oiled with a remaining cocoa butter content of 20-22 percent. Cocoa butter often passes through a process of alkalization also called "Dutch processing", in which the pH value is increased from 5.3-5.8 to 6.8-8.1. This increase reduces bitterness, improves solubility, and intensifies color. However, naturally high contents of potentially healthy flavonoids in cocoa powder are substantially reduced by the alkalization process. Cocoa powder is used as beverage, as baking or chocolate ingredient (MILLER et al., 2008).

Furthermore, cocoa beans are the most important ingredient in chocolate production. However, there are different ways of chocolate production. A classical way is to mix the cocoa liquor with either icing sugar or granulated sugar and if desired with milk powder, cocoa butter and lecithin. The ingredients are mixed, refined and conched. Afterwards, chocolate mass is tempered, molded and packed. Chocolate can be either made from bulk or from fine and flavor cocoa beans. Chocolate

made of bulk cocoa obtains mainly low prices and is made with more different ingredients (e.g., milk chocolate). In contrast, chocolate of fine and flavor beans is more expensive and often have a high cocoa content (e.g., LIEBEREI AND REISDORFF, 2012; STOLL, 2010).

Most recently, also the cocoa seed shell is used, for example for tea infusions. As it also contains theobromine (approx. 0.8%), it can be beneficial for health (LIEBEREI AND REISDORFF, 2012). As stated earlier, in some countries like Brazil cacao pulp is used for beverages, jellies, jam etc. (STOLL, 2010).

1.4 International cocoa market – Fine vs. bulk cocoa

Production and demand of raw cocoa beans have strongly increased during the last 200 years. In 1840, approximately 14,000 tons of raw cocoa beans were produced worldwide while in 2005 global production was at 3.9 million tons (LIEBEREI AND REISDORFF, 2012). Furthermore, the importance of cocoa producing countries shifted drastically more than once in history. Changes were mostly caused by pest and disease outbreaks or by political instability in the respective countries. At the beginning of the 20th century, most cacao was grown in Latin America, whereas today, main production countries are located in Africa and Asia (STOLL, 2010).

Traditionally, Brazil was a major cacao growing country. As the cacao tree is native to the Amazonas basin, cacao fruits were harvested of wild growing trees in the rain forests. Still, some of those remnant production sites exist, but cacao of this area has become a specialty. Since cacao seeds were taken from the state of Pará in the Amazonas region to the state of Bahia in 1746 AD, the main growing area in Brazil established there with 85 percent share of total Brazilian production (PEREIRA et al., 1996). Still in the 1980s, Brazil was the 2nd largest producer of raw cocoa beans behind Ivory Coast in the world (WEGNER, 2001). However, the introduction of witches' broom disease in 1989 from plantations in the Amazonas region to Bahia resulted in a drastically cacao production decline. Hence, Brazil turned to a cocoa importing country to satisfy the demands. Until today, the cacao production in Brazil recovers slowly even though more resistant plant material is available (PEREIRA et al., 1996).

Today, world raw cocoa beans production is at 4.2 million tons with a value of approximately 11.8 billion US$. About 40 to 50 million people depend on cacao farming for their income (BEG, 2017). Africa contributes approximately 71 percent of the global cocoa production, South America only 16 percent. Ivory Coast alone produced 2 million tons raw cocoa beans, Ghana 1 million tons, Indonesia 600,000 tons, Nigeria 330,0000 tons, Cameroon 300,000 tons, Brazil 240,000 tons, Ecuador 235,000 tons, and Peru 135,000 tons (all values approximated) in 2018 (FAO, 2018). In the past, the main cocoa consuming regions were North America and Europe. During the last two decades, further

consumption countries have gained more importance due to a higher income and better living conditions, such as China and India. Furthermore, cocoa producing countries, e.g., in Africa, South America and Asia, have begun to produce and to consume cocoa and chocolate products themselves to gain additional value (SQUICCIARINI AND SWINNEN, 2016).

In the 20th century, Criollo varieties were often replaced by Trinitario cultivars in many parts of the world as Criollo cacaos did not pay off for the farmers. Only after international appreciation of this variety and the introduction of the trade classifications "bulk" vs. "fine and flavor" cacaos, combined with surcharges, Criollo cultivation could be stabilized. Therefore, today the international cocoa market differentiates between "bulk" and "fine and flavor" raw cocoa beans. The criterion for the classification is the genotype of the cacao tree. Fine and flavor cocoa beans derive from Criollo or partly Trinitario cultivars, bulk cocoa beans derive from Forastero and, depending on the aroma and flavor traits, partly from Trinitario cultivars. Moreover, Nacional cocoa beans of Ecuador, that genetically belong to the Forastero cultivars, are classified as fine and flavor cocoa for its unique flavor traits (LIEBEREI et al., 2010). However, about 85-90 percent of the global cocoa production are classified as bulk cocoa, whereas the remainder is predominantly fine and flavor cocoa from Trinitario or Nacional cultivars and only less than 1 percent only from Criollo varieties (LIEBEREI et al., 2010). Due to the increasing consumer interest in healthy and tasteful cocoa products, also the demand of fine and flavor cocoa is increasing constantly (GOCKOWSKI et al., 2011).

Prices for raw cocoa beans depend on the international stock market as it is traded as a commodity good at the commodity futures exchange in London (International Financial Futures and Options Exchange, LIFFE) and New York (Coffee, Sugar and Cocoa Exchange Inc.). The price level of cocoa is usually applied for commodity cocoa, whereas for fine and flavor cocoa premium prices are paid that can be up to four times the price of standard bulk cocoa (TROGNITZ et al., 2011). In order to facilitate the classification for the cocoa buyers, the International Cocoa Organization (ICCO) holds meetings of an international expert panel that recognizes countries as fine and flavor cocoa exporters at irregular intervals. The last meeting took place in 2016 when 23 countries were recognized as exporters of fine and flavor beans. However, not all countries are recognized with 100 percent of their export quantities. Depending on the market share and product quality, premium prices vary. Furthermore, only export quantities are considered. In case of a strong local cocoa and chocolate market, these cocoa qualities are not taken into consideration (ICCO, 2016).

Certification schemes of cocoa products in the cocoa value production chain are constantly increasing. For raw cocoa beans certification of organic qualities, fair-trade as well as UTZ/Rainforest Alliance are the most common. On organic cacao farms, no conventional pesticides or fertilizer may be used. Other certificates allow so-called "integrated pest management" where conventional

products may be used if not avoidable. However, the most important interest of the labels in Africa is to prohibit child slavery and child labor in cacao plantations which is still a common phenomenon in West Africa. Also, compliance of labor standards as well as forest conservation are aims of the labels. Fair-trade, for example, aims at improving the economic, ecologic and social situation of the cacao farmers through more fair-trade relations (BDSI, 2020). The market share of certified cocoa is constantly increasing, but seen at a global scale still very low. In 2005, the market share of organic cocoa beans was less than 0.5 percent with more than 70 percent of organic raw cocoa beans coming from Latin America. Fair-trade is even lower with 3,901 tons in 2005. However, several countries in the meantime have converted part of their production to organic farming (ICCO, 2006). By selling certified raw cocoa, cacao farmers are paid a premium depending on the product quality.

1.5 Cacao cultivation in the department San Martín, Peru – The role of UNODC in cacao cultivation

Although *Theobroma cacao* L. originates from the Eastern slopes of the Andes, inter alia on the Peruvian territory, it was not cultivated in Peru until the 18th century (ZHANG et al., 2006). Starting from the 1960-70s cacao was pushed out of many parts of Peru for increased cultivation of coca (*Erythroxylum coca* Lam.) that was mainly used for the illicit manufacture of cocaine (ESPINOZA HARO, 2008). Between the 1980s to the 1990s, coca cultivation reached its peak especially in the areas of Bajo Huallaga and Huallaga Central, which transformed Peru to the country with the highest coca production in the world. In the mid-1990s, coca production had spread all over Peru with a production potential for cocaine of 460 tons per year. The production of coca was related to an armed conflict of two terrorist organizations (Partido Comunista del Perú Sendero Luminoso and, to less extent, Movimiento Revolucionario Túpac Amaru) that aimed at destroying the democratic structures in Peru, which caused severe torment for the population of Peru due to drug violence. Therefore, from 1993 onwards, the government of Peru began to fight against the illegal cultivation of coca (ESPINOZA HARO, 2008).

Already in 1986, UNODC (United Nations Office on Drugs and Crime) and later on also other NGOs (e.g., USAID – US governmental organization; PRODATU – a German cooperation; PRODAPP – a program of the European Union) entered Peru to initiate and implement an alternative development which aimed at replacing coca production by alternative crops, including cacao. However, until today coca continues to be cultivated in Peru for the illicit production of cocaine (ESPINOZA HARO, 2008; Fig. 8). In 1986, UNODC imported Trinitario cacao seeds (albeit with strong Forastero characteristics and therefore classified as bulk cocoa quality) from Brazil and Colombia to the regions of San Martín,

Huánuco, and Ucayali and established plantations according to the recommendations of cocoa specialists from Colombia, Brazil, and Ecuador.

Figure 8: Coca cultivation in San Martín, Peru and its effects: **a** coca shrub in a cacao plantation; **b** coca plantation with some cacao trees; **c** street patrol in a dangerous mountain area where coca is still cultivated.

Furthermore, UNODC started to found first cocoa cooperatives, e.g., the "Cooperativa Agroindustrial Tocache (CAT)" in Tocache. Cacao farmers were encouraged to become members of the cooperatives to benefit from shared knowledge, equipment, joint fermentation and drying facilities, and quality standardization along with joint cocoa sales and distribution. However, in 1989, the fungal disease frosty pod reached Peru for the first time and rapidly spread to all cacao growing areas during the following years, which caused severe harm to the entire cacao-based approach. Therefore, UNODC set phytosanitary action plans in force to protect the participating cacao farmers. Apart from importing seed material from abroad from 1987 onwards, the project started a program for the recovery of the Peruvian gene pool by collecting genetic material in the main river basins of the rivers Amazonas, Ucayali, Urubamba, Huallaga and Marañón. Together with all main universities in the Amazonian part of Peru and some cacao producer organizations, the genetic cacao material was installed in cacao gene banks as well as in so-called "clone gardens" from which plant material for grafting could be obtained. In 1994, UNODC started to change their strategy towards the cultivation of the productive and pest-tolerant cacao clone CCN-51 from Ecuador. This clone was distributed to most plantations in the departments of Huánuco, San Martín and Ucayali, whereas only few former cacao trees remained in the plantations. However, as CCN-51 classified as bulk cocoa due to its acid, bitter, and astringent aroma profile, UNODC again changed the strategy for cacao cultivation in Peru towards fine and flavor cocoa varieties that already showed increasing demand and gained a premium on the regular stock market price of raw cocoa beans for the cacao producers. Therefore in 2007, UNODC started identifying and studying suitable cacao varieties and explored adequate plantation designs including agroforestry systems. Already in 2008, Peru was recognized as a 100 percent exporting country of fine and flavor cacao by the International Cocoa Organization (ICCO) (albeit in 2016, Peru was ranked with 75 percent fine and flavor cocoa share, which seems

more realistic, ICCO, 2016). From 2009 onwards, UNODC and USAID supported the cultivation of eight selected Trinitario clones (ICS-1, ICS-6, ICS-39, ICS-60, ICS-95, TSH-565, UF-29 and UF-613) and one Forastero clone (IMC-67; for pollination reasons) in polyclonal agroforestry systems in the departments of San Martín and Huánuco (Fig. 9).

Figure 9: Clones selected by UNODC (ICS-1, ICS-6, ICS-39, ICS-95, IMC-67, TSH-565 and UF-613, with ICS-60 and UF-29 missing) for cultivation in the states of San Martín and Huánuco, Peru. Two fruits of each clone are shown.

These clones were selected based on their productivity (2–3.5 t ha^{-1} $year^{-1}$ in well maintained plantations with a planting density of 1,111 individuals ha^{-1}), pest resistance, and flavor and aroma characteristics along with their availability for propagation (GÓMEZ ALIAGA et al., 2014; unpublished presentation ROBERTO GÓMEZ ALIAGA, UNODC; pers. comm. GREGORIO SAEZ MOYA, UNODC). Furthermore, a selection criterion was a moderate heavy metal uptake such as cadmium, that frequently occur in soils in South America and is known to be accumulated by the cacao tree in the seeds (ZUG et al., 2019). The project recommended diversifying the clones on each farm to reduce the risk of yield loss from diseases or pests. Moreover, fine and flavor cacao has usually been planted in mixed agroforestry systems to provide the farmers with other sources of income. To avoid accidental mixing with CCN-51 bulk cacao, the Trinitario clones were planted separately from CCN-51 plantations. Also, new fermentation facilities were constructed to ensure separate handling of the Trinitario clones and CCN-51, as a mixture could on the one hand endanger the fine and flavor status of Peru and on the other result in lower sales prices for the farmers (GÓMEZ ALIAGA et al., 2014; unpublished presentation ROBERTO GÓMEZ ALIAGA, UNODC; pers. comm. GREGORIO SAEZ MOYA, UNODC).

In parallel to the activities of cultivating the nine international Trinitario clones, further investigation on local fine and flavor varieties has been done by the UNODC to, in the long run, establish plantations that produce cacao with a unique flavor profile using local cacao varieties and adapted farm management practices. At the moment, only single trees of each promising cultivar are available as all other trees were felled in favor of the clone CCN-51. Therefore, evaluation and propagation of the local varieties is pronouncedly time-consuming (pers. comm. GREGORIO SAEZ MOYA, UNODC).

Due to the fact that the Trinitario clones were introduced for the first time to Peru or recovered from old gene banks in Peru, the knowledge regarding adequate post-harvest treatments is lacking. This undermines the aim of the UNODC to strengthen the position of Peru as high-quality cocoa producer on the world market. Therefore, this study aims at filling this gap in scientific knowledge. Additionally, adapted farm management practices that allow for high fruit set and yield along with a favorable profile of methylxanthines and polyphenols for the area of Tocache are so far not available. Most methodologies were copied from other cacao growing areas in South America, irrespective of the fact that soils, climate regime, and herbal and shade plant compositions are different in the study area. To provide Peruvian farmers with the required information, this study analyzed a huge array of different cultivation methods to disentangle the main drivers of yield and quality parameters of *Theobroma cacao*.

1.6 Study area and sites

This study was carried out in the province of Tocache in the department of San Martín in the northeastern part of Peru (8°11'19.565"S, 76°33'31.626"W). The area is located in the Huallaga River valley at the eastern foothills of the Andes mountains at an altitude of 497 m above sea level and belong to the Amazonas basin. In the past, the area was almost completely covered by tropical lowland, sub-montane, and montane rainforests. In the meantime, it has largely been converted to crop fields, pastures, and agroforestry systems by slash-and-burn activities. The main soil types in the area are Inceptisols (Dystrudepts; PROGRAMA DE DESAROLLO ALTERNATIVO TOCACHE UCHIZA et al., 2008). Climate is typical for tropical lowlands (SOBERANIS et al., 1999). Mean annual precipitation at the town Tocache Nuevo is at 2,400 mm with a pronounced rainy season between October and March. Mean annual temperature is 24.7 °C, and mean relative humidity is 70–80%, usually reaching 100% during the day (PROGRAMA DE DESAROLLO ALTERNATIVO TOCACHE UCHIZA et al., 2008; SOBERANIS et al., 1999).

The experimental sites were located within a radius of 15 km from Tocache. Overall, 14 farms were evaluated regarding farm management aspects, situated mainly north, north-west and north-east of the town Tocache (Fig. 10). Farms were selected with respect to the availability of old local varieties as well as CCN-51 within the same plantation. As the CCN-51 trees are genetically identical, they served as reference trees because the traits of the local trees were all different and unknown. Furthermore, a criterion for the selection of farms were ripe, available fruits for the analysis of flavor relevant substances within the seeds. The on-farm investigations were carried out in June, 2014.

The two fermentation experiments were located in different post-harvest sites (see Fig. 10: FS 1 and FS 2) of two cacao cooperatives. Experimental single-clone and mixed micro-fermentations were carried out at the post-harvesting facilities of the cooperative CAT (Cooperativa Agroindustrial

Tocache, since 2019 named “Cacao Tocache”) approximately 3 km from the center of Tocache in north easterly direction in the hamlet “Bajo Almendras” (S8°09'59.1", W76°31'43.5") (see Fig. 10: FS 1) in 2011 and 2012. The premises of the cooperative consisted of an at that time very badly managed clonal garden as well as separate fermentation boxes for CCN-51 and fine and flavor cocoa along with solar pre-drying and drying facilities.

The commercial-scale fermentations with Trinitario mixtures were carried out in the vicinity of the village “Alto Bambamarca” in the province of Tocache, San Martín, Peru (S8°8'40.661", W76°35'8.804") (see Fig. 10: FS 2) between April and June 2013. The fermentation and drying modules were constructed in 2012 by CITEcacao (Centro de Innovación Tecnologia del Cacao, Peru) and managed by the cacao cooperative Cooperativa Agroindustrial ASPROC-NBT Ltda. (Bambamarca). This site consisted of a cacao plantation nearby, separate fermentation facilities for CCN-51 and fine and flavor cocoa as well as pre-drying and drying facilities.

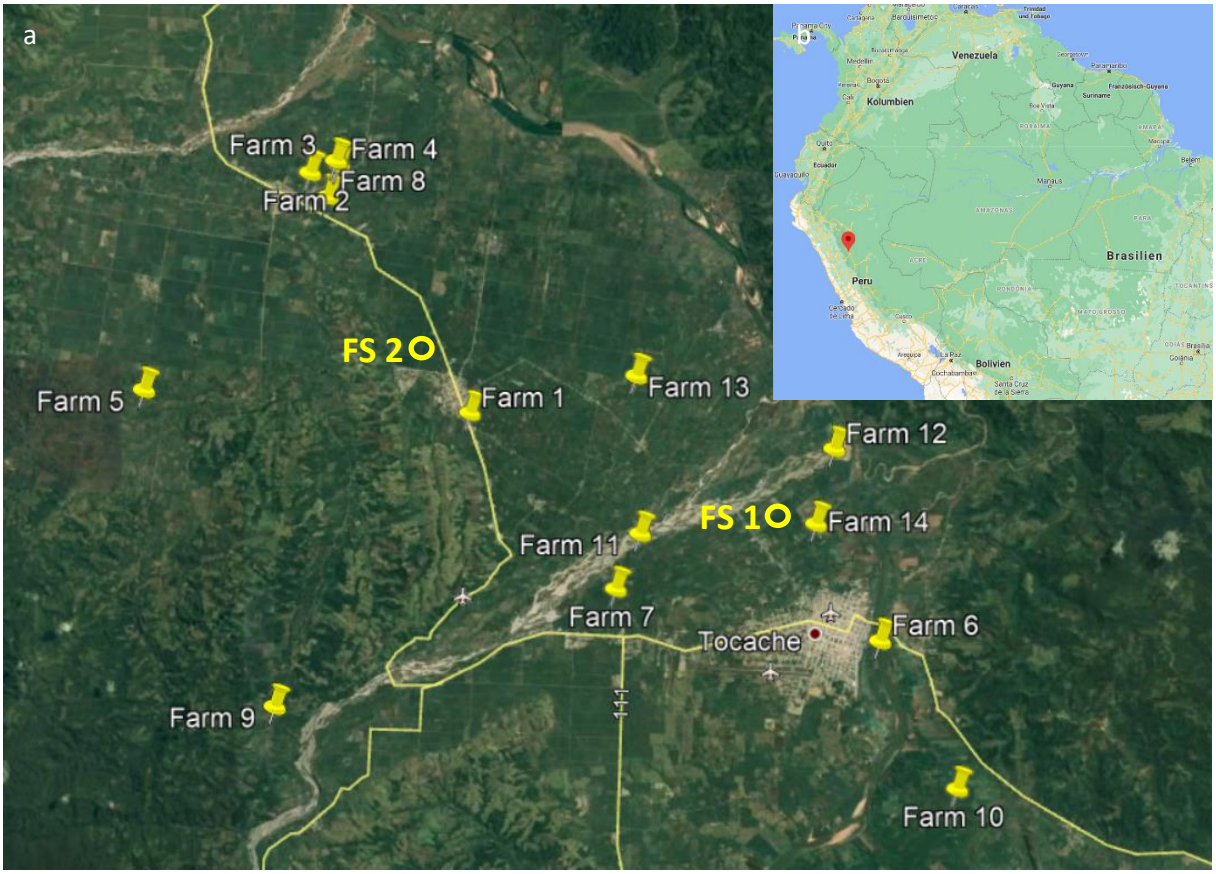

Figure 10: Study area with **a** detailed map of the positions of the investigated cacao farms (Farm 1 to 14, yellow pins) as well as the two fermentation sites (FS 1 and FS 2, yellow circles) in the surroundings of the town Tocache nuevo (Tocache) in the province of Tocache, department San Martín and **b** overview map of Peru in South America.

2 PLANT DIVERSITY EFFECTS ON CROP YIELD, PATHOGEN INCIDENCE, AND SECONDARY METABOLISM ON CACAO FARMS IN PERUVIAN AMAZONIA

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2.1 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, 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 farms in Peruvian Amazonia were analyzed. The 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. These 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.

2.2 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 (LETOURNAEU 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, 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 et al., 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 cacao yield overall and per plant (DEHEUVELS et al., 2012; see also OFORI-BAH AND ASAFU-ADJAYE, 2011) and 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, dried 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 this 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 twenty 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 single 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. 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 cacao yield, pathogen incidence, and plant secondary compounds such as polyphenols and methylxanthines. In particular, the following questions were considered: (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 cacao 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.

2.3 Materials and Methods

2.3.1 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). 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 by the UNODC in order to maintain the consisting plant material. For novel cacao plantings initially, to increase the project's likelihood of success, 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 et al., 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 2,400 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 et al., 2008; SOBERANIS et al., 1999).

2.3.2 Study design

In the study area, 14 cacao farms were randomly selected 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 in Annex, Tab. 6).

2.3.3 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, all shade trees were counted and identified 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* infested pods were counted, 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. Length and circumference of the pods were measured. For biochemical analyses, unfermented cacao seeds were removed from the pods and air-dried.

Insolation in percent was estimated visually on-site, and shading of the cacao tree and of 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 (Tab. 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.

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
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)

2.3.4 Laboratory analyses

2.3.4.1 Chemicals and standards

Unless otherwise specified, all chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). Epicatechin, catechin hydrate, cyanidin-3-arabinoside, cyanidin-3-galactoside,

theobromine, and caffeine were purchased from Sigma. Water was deionized in an Elga water purification system (PURELAB Option, Elga, UK).

2.3.4.2 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^3 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 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.

2.3.4.3 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 510 H, 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 transferred to 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).

2.3.4.4 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 mesh size 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.

2.3.4.5 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} $K_4[Fe(CN)_6]$ * $3H_2O$) was added followed by clarifying with 0.2 mL of Carrez II solution (300 g L^{-1} $ZnSO_4$ * $7H_2O$). 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.

2.3.4.6 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 $CaCl_2$ 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 to 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).

2.3.5 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, environmental data were fitted 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). Only variables with a *p*-value < 0.05 were included.

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 ($D = 1 - \sum_{i=1}^{S} p \frac{2}{i}$ 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. Generalized linear mixed models (GLMM) were used 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 the 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 et al. 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, it was 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 Akaike information criterion (AIC) values.

2.4 Results

2.4.1 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. 11a) 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 considerably lower at a higher cover of the herb layer.

NMDS (Non-metric multidimensional scaling) of shade tree composition (Fig. 11b) 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 phytosanitary pruning. As alpha diversity of shade trees is directly managed by the farmers, possible drivers of the shade tree diversity were not tested.

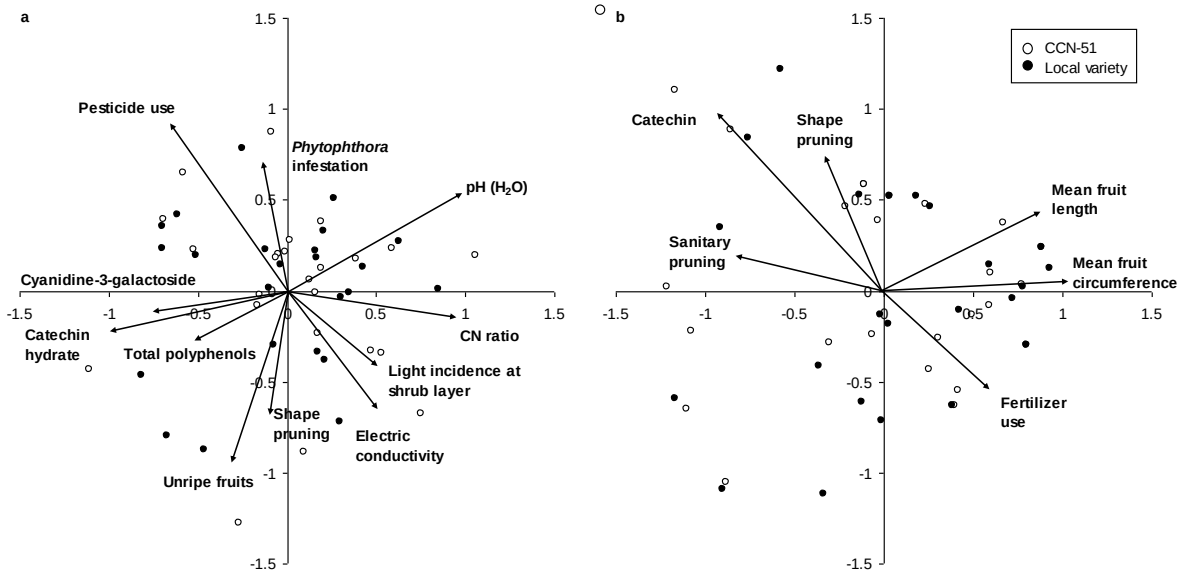

Figure 11: Correlations of species composition in **a** herb and shrub layer and **b** shade tree layer with soil, management, pathogen incidence, crop yield, and crop quality parameters according to NMDS. Only significant correlations ($p < 0.05$) are displayed. Stress values: a: 0.11; b: 0.10.

In general, these results show that biodiversity did not result in a reduction of cacao yield. No evidence was found 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 ($p = 0.0129$, Tab. 2), lower when *M. roreri* incidence decreased ($p = 0.0192$), 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 (Tab. 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. 12a). 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).

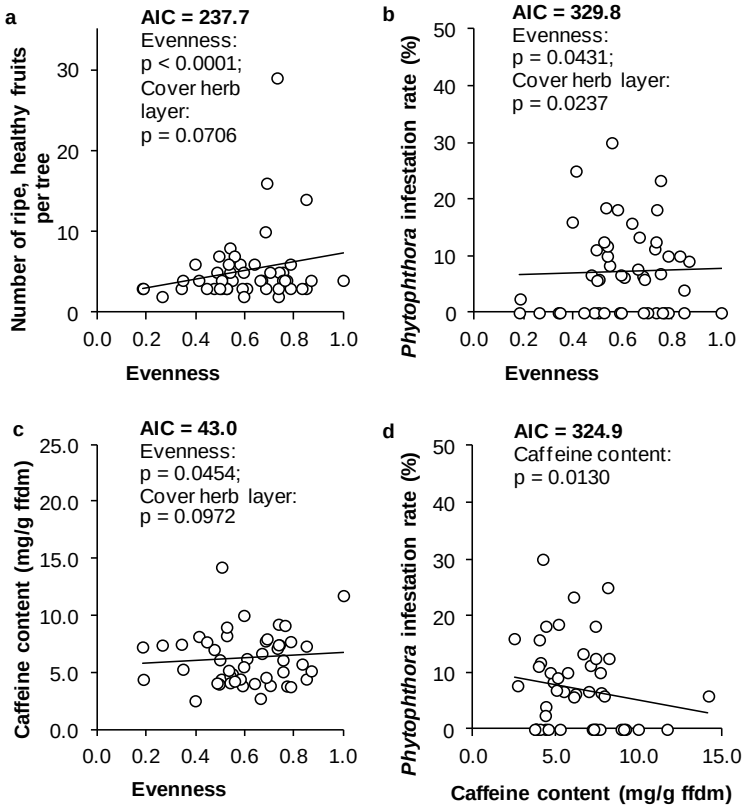

Figure 12: Correlations of **a** the number of ripe fruits per tree (glmer (Fruit number~Evenness + Cover herb layer + (1|Farm/Plot pair/Plot), family = "poisson"), **b** *Phytophthora* incidence (lme (*Phytophthora*~Evenness + Cover herb layer, random = ~1|Farm/Plot pair/Plot), and **c** caffeine content 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.

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 (Tab. 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.

2.4.2 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 (Tab. 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 with cacao trees and 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. 12b). Soil parameters and genetic background did not affect *Phytophthora* infestation (Tab. 2). The infestation rate with *M. roreri* was more pronounced at high evenness of the herb and shrub layer (Fig. 13a). Cacao trees with higher epicatechin content showed lower levels of this fungus (Fig. 13c). In addition, fertilizer use (irrespective of type) significantly decreased *M. roreri* incidence (AIC = 328.7). Again, CCN-51 did not differ from the local varieties in terms of *M. roreri* infestation (Tab. 2).

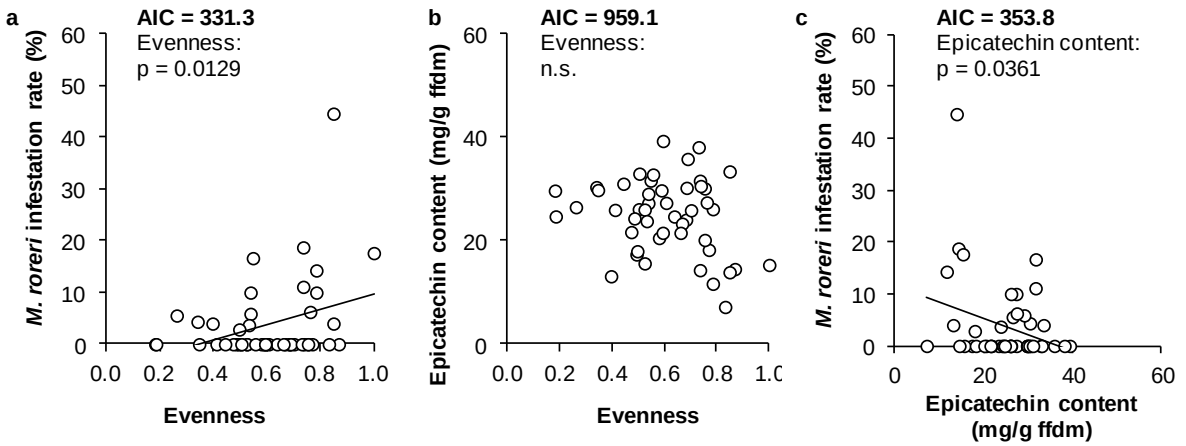

Figure 13: Correlations of **a** *Moniliophthora roreri* incidence (lme (*Moniliophthora*~Evenness, random = ~1|Farm/Plot pair/Plot) and **b** epicatechin content with evenness (lme (Epicatechin~Evenness, random = ~1|Farm/Plot pair/Plot); **c** correlation of epicatechin content with *M. roreri* incidence (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.

Local varieties showed a marginally higher rate of infestation with *M. perniciosa* in comparison to CCN-51 (Tab. 2). Both total phenolic compounds and cyanidin-3-glycosides were higher at higher levels of infestation with witches' broom (Fig. 14c). 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 cyanidin-3-glycoside content ($p = 0.0246$) were related to a higher infestation rate (AIC = 246.2), which points to certain 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. 14a).

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.

2.4.3 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, $p = 0.0145$, phytosanitary pruning, $p = 0.0048$, electric conductivity, $p = 0.0009$, and *M. roreri* incidence, $p = 0.0132$, AIC = 915.2) and cyanidin glycosides increased with the basal area of shade trees (Fig. 14b). 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 after shape pruning ($p = 0.0175$, combined with a marginally significant effect of phytosanitary pruning, AIC = 697.3). Cyanidin 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 cyanidin glycosides in cacao seeds ($p = 0.0415$, AIC 751.6).

Table 2: Fruit number and size, pathogen incidence, plant secondary compounds in dried 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.	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 in dried cacao seeds (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)
Cyanidin-3-galactoside (mg/g ffdm)	0.41 (0.04)	0.45 (0.06)
Cyanidin-3-arabinoside (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)

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 phytosanitary 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. 12c). 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).

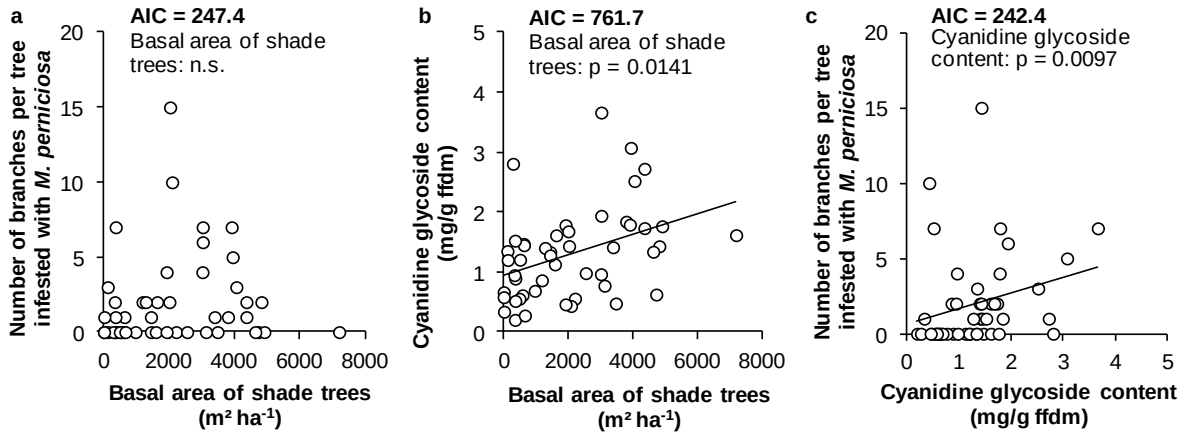

Figure 14: Correlations of **a** *Moniliophthora perniciosa* incidence (lme (*M. perniciosa*~Basal area of shade trees, random = ~1|Farm/Plot pair/Plot) and **b** cyanidin glycoside content with basal area of shade trees (lme (Cyanidin glycoside~Basal area of shade trees, random = ~1|Farm/Plot pair/Plot); **c** correlation of cyanidin glycoside content with *M. perniciosa* incidence (lme (*M. perniciosa*~Cyanidin 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.

2.5 Discussion

The 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), no negative impact of herb diversity on yield was found 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 (GUIDOIN 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 these data, which is a novel aspect in biodiversity studies (SAMEDANI et al., 2014).

2.5.1 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; DAGHELA 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.

Clear evidence was found 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 this 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 this 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 cacao yield. While chemical fertilizer usage 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 this 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 a 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 the models (see Fig. 12a), 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, the 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. 11a). 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 cacao production. Consequently, the biodiversity effects in this 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; SOBARANIS 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 these 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 cacao 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 this 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. 11b) 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 cacao 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.

2.5.2 Impact of biodiversity on pathogen incidence

The 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 this 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 cyanidin 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 this study, the more common *Phytophthora* was down-regulated in favor of the less common *M. roreri,* which is characterized by 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.

2.5.3 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, a combination of different plant secondary compounds was found, 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 cyanidin 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 cacao production.

2.5.4 The relevance of the genetic background

In contrast to the 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 cyanidin glycosides were slightly higher and those of other polyphenols lower in local cacao compared to CCN-51, but neither significantly (see Tab. 2). In terms of phenolic content the results matched with other studies (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 Tab. 2).

There was a significant difference towards a higher total pod number per tree as well as towards unripe fruits in local cacao trees. Furthermore, a not significantly greater infestation with *Moniliophthora* species in local varieties was found 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 the data of this study.

2.6 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, overall negative consequences of the shade tree layer on the number and size of healthy, ripe pods were found. 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 cacao 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 phytosanitary 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.

3 INFLUENCE OF PULP PRE-CONDITIONING ON FERMENTATION AND RAW COCOA BEAN QUALITY IN TRINITARIO CLONES (*THEOBROMA CACAO* L.)

3.1 Abstract

In cacao, fermentation is crucial the formation of flavor precursors and thus for the final product quality. Fermentation procedures should be adapted to genotype and environmental conditions. To better control the process, fermentation can be modulated by previous pulp pre-conditioning. Therefore, the impact of pod storage, pulp drainage, and inoculation with starter cultures on the fermentation process, the contents of free amino acids and polyphenols and the sensory characteristics of a typical Trinitario clone mixture in Peru were explored. This study revealed significant differences among treatments. Pulp drainage led to a significantly more rapid increase in fermentation temperature than pod storage, and fermentation time tended to be shorter. Pod storage resulted in higher contents of acidic amino acids, asparagine, and epicatechin compared to the other treatments. Accordingly, higher fermentation temperature led to a lower content of amino acids and polyphenols in raw cocoa beans which resulted in lower acidity, astringency, and bitterness. The findings provide evidence that pod storage leads to increased and pulp removal to reduced bitterness and astringency in the clone mixture used. Based on the results of this study, pulp pre-conditioning can optimize fine cacao quality in accordance with consumers' preferences.

3.2 Introduction

Cacao production is of pivotal importance for more than 5 million smallholder farmers in the tropics (WORLD COCOA FOUNDATION, 2014) as well as for chocolate companies located mainly in industrialized countries. Accordingly, it is one of the most important cash crops worldwide with continuously growing global production (2012: 4.8 10^6 t; production increase 2008-2012: 13% $year^{-1}$; WORLD COCOA FOUNDATION, 2014), and 40–50 million people in producing and processing countries depend on it for their livelihoods (KONGOR et al., 2016).

Along with genetic background (NIEMENAK et al., 2006; KONGOR et al., 2016) and farming conditions (e.g., soil, shade management; see ELWERS et al., 2009 and KIECK et al., 2016), post-harvest treatments—pulp pre-conditioning, fermentation, and drying—determine the quality of the raw cocoa beans (e.g., APROTOSOAIE et al., 2016; KONGOR et al., 2016). During fermentation and drying, aroma precursors are produced that are essential for the typical chocolate aroma that ultimately

develops in the Maillard reaction during roasting (e.g., GIACOMETTI et al., 2015). At the same time, bitterness and astringency are reduced (OLIVIERO et al., 2009; KONGOR et al., 2016). Fermentation methods, duration, mixing intervals, and different treatments are adjusted based on the genetic background of the beans and the environmental conditions (e.g., SALTINI et al. 2013).

For fermentation, the harvested cacao pods are opened, and the seeds embedded in the mucilaginous pulp are placed in fermentation vessels (e.g., wooden boxes). Cacao fermentation generally consists of three phases (e.g., DE VUYST AND WECKX, 2016): (1) anaerobic yeast phase (hours 0–48 of fermentation), in which pulp sugars are transformed to ethanol and pulp is liquefied by yeasts; (2) microaerobic lactic acid bacteria phase (hours 24–72), in which lactic acid is produced by lactic acid bacteria; and (3) aerobic acetic acid bacteria phase (hours 48–112), in which ethanol is transformed to acetic acid by acetic acid bacteria. Oxygen enters into the fermentation mass through gaps after the liquid pulp is drained off. For further aeration, turning of the mass is necessary. The acidification is accompanied by a steep increase in temperature up to 50 °C, which, together with acids penetrating the seed's micropyle, leads to the embryo's death and the subsequent destruction of cell compartmentation. Proteolytic cleavage of storage proteins and sugars and oxidation of polyphenols follow, with the latter being visible by browning of the seeds.

In general, bulk cocoa requires a longer fermentation time, up to 8 days, compared to fine and flavor cocoa, with some Criollo types taking just 2–3 days to ferment (AFOAKWA et al., 2008; SALTINI et al., 2013). In contrast to bulk cacao, fine and flavor cacao fermentation aims both to produce the typical chocolate flavor through proteolytic degradation of storage proteins and to conserve the clone-specific aroma components leading to fruity, flowery, nutty or caramel notes, which derive from the cacao pulp (KADOW et al., 2013) and also from seeds (APROTOSOAIE et al., 2016). However, fermentation procedures for fine and flavor cacao clones in particular from South American are not yet available.

To optimize fermentation, different pre-conditioning treatments of the cacao pods and the fermentation mass have been proposed (MEYER et al., 1989; BIEHL et al., 1990; PASS, 1996). (1) Pod storage: To decrease the acidity of raw cocoa beans, pods can be stored prior to fermentation (MEYER et al., 1989; PORTILLO et al., 2007). The storage reduces the pulp volume and results in a less pronounced growth of yeasts and lower final content of acetic acid in the fermented raw cocoa beans (MEYER et al., 1989). Also, the content of minerals and polyphenols and the chemical composition of fermented raw cocoa beans (AFOAKWA et al., 2012a, 2013) along with the fermentation index (AFOAKWA et al., 2012b) are modified by pod storage. (2) Seed pre-drying and pulp drainage: These are equivalent methods to counteract strong raw cocoa bean acidification by reducing pulp volume and sugar content (SCHWAN AND WHEALS, 2004) but may lead to irregularities in

the fermentation process (BIEHL et al., 1990). (3) Inoculation: Amending the fermentation mass with starter cultures is a common approach to better control the fermentation process and the final quality of the raw cocoa beans (SCHWAN AND WHEALS, 2004; DE MELO et al., 2012; KRESNOWATI AND FEBRIAMI, 2016; recent review by DE VUYST AND WECKX, 2016). Furthermore, a method similar to inoculation has been studied, involving the direct use of fermentation enzymes (BINH et al., 2012), but for on-farm application this has been seen as too complex and costly (SCHWAN AND WHEALS, 2004). Whether the treatments described above are also appropriate for fermentation of fine and flavor cacao in South America has not yet been explored. Therefore, the model experiments in Tocache, San Martín, Peru were performed with Trinitario clones that were previously selected according to their productivity, pest resistance, flavor and aroma characteristics as well as their availability for propagation (GÓMEZ ALIAGA et al., 2014). In addition, a comparison of different pre-conditioning treatments has so far rarely been conducted (PASS, 1996).

This study explores the impact of different pre-conditioning treatments (pod storage, pulp drainage, inoculation with starter cultures) on temperature development, formation of free amino acids, and decrease in polyphenols, and the resulting effects on the sensory characteristics of a common mixture of selected Trinitario clones (ICS-1, ICS-6, ICS-39, ICS-95, TSH-565, and UF-613) and IMC-67. Treatments were selected and adapted according to practical requirements of the local cacao farmers and cooperatives to facilitate local adoption of the methods. For each treatment, experimental fermentations were performed and the fermentation process and the quality of fermented dried raw cocoa beans analyzed. Overall, we aimed to adapt the fermentation process to obtain a high-quality product that could lead to a potentially higher market price for the farmers.

In particular, we assessed the following questions: (1) Do the treatments influence the course of fermentation in terms of temperature increase and acetic and lactic acid formation? (2) Do the treatments influence the contents of free amino acids and polyphenols in the beans and is there a relationship to the temperature regime? (3) Do the treatments, the temperature profile, or the content of amino acids and polyphenols influence the sensory characteristics of the raw cocoa beans?

3.3 Materials and Methods

3.3.1 Study site

The experimental site was located in the vicinity of the village Bambamarca in the province of Tocache, San Martín, Peru (S8°8'40.661", W76°35'8.804"). The fermentation and drying modules were constructed in 2012 by CITEcacao (Centro de Innovación Tecnologia del Cacao, Peru) and managed by the cacao cooperative Cooperativa Agroindustrial ASPROC-NBT Ltda. (Bambamarca) (Fig.

15a). The fine and flavor fermentation boxes were located in a brick house with a metal roof and mesh in the upper part of the building for ventilation. Four fermentation rows of three or four fermentation boxes each were used. Each box had a dimension of 0.7 m x 0.7 m x 0.7 m (0.343 m^3) and was insulated with 1 cm thick polystyrene panels at the long- and broadsides. The fermentation site was used for the first time during these fermentations. Two 6-day fermentation runs with Trinitario cacao were performed prior to the experimental fermentations to promote establishment of favorable microorganisms. The first box of each row contained 25 holes at the bottom with a diameter of 0.8 cm each to allow for pulp drainage during the first fermentation phase. The other boxes in each row served for mixing and aerating the fermentation mass (two to three times per fermentation run).

For drying, existing drying compartments were used, one with a roof and sliding trays (Fig. 15b) and another with a concrete floor and without roof (see below).

3.3.2 Study design

Cacao pods for fermentation were obtained from cacao farmers participating in the United Nations Office of Drugs and Crime (UNODC) project “Cacao instead of coca” in the province of Tocache. A defined mixture of the clones ICS-1, ICS-6, ICS-39, ICS-95, IMC-67, TSH-565 and UF-613, representing their mean occurrence in the field (Tab. 3) was used. Identification of clones was supported by agrarian agronomists and technicians of UNODC.

Four different pulp pre-conditioning treatments were run in parallel: (1) 3-day storage of cacao pods, (2) pulp removal, (3) inoculation with yeast and acetic acid bacteria preparations, and (4) control. The set-up was replicated four times over 4 consecutive weeks between April and May 2013.

Treatment 1: Pod storage

Pods were sun-dried in single layers on polyethylene tarpaulins on a concrete floor for 3 days. After storage, pods were opened, the clones mixed according to Tab. 3, and the seeds placed in a fermentation box (Fig. 15c).

Treatment 2: Pulp removal

Before transfer to the fermentation box, seeds with pulp were put in a net with mesh size of 1 x 1 cm. Each time, 20 L of mass was treated. The net was hung up on a joist and wrung three revolutions for 1 min for pulp drain off. Afterwards, the seeds with the remaining pulp were put into a fermentation box.

Table 3: Characteristics of clones used in the fermentation experiment. Mean (standard error).

Clone	**ICS-1**	**ICS-6**	**ICS-39**	**ICS-95**	**IMC-67**	**TSH-565**	**UF-613**
Percentage per fermentation box (%)	16.70 (1.23)	14.24 (1.10)	14.45 (1.26)	9.10 (0.55)	11.25 (0.87)	32.71 (2.20)	1.54 (0.56)
Fruit pulp traits							
Fruit pulp/seed ratio (n = 3 x 50 seeds)	0.69 (0.07)	0.81 (0.15)	0.68 (0.07)	0.71 (0.02)	0.77 (0.05)	0.84 (0.05)	0.58 (0.58)
Moisture reduction of fruit pulp: fresh/pre-stored pods (%) (n = 3 x 50 seeds)	30.75 (13.49)	20.99 (20.99)	44.99 (7.95)	48.25 (5.37)	19.99 (6.04)	32.50 (3.15)	-
Brix value of fruit pulp (fresh; n = 5 fruits)	16.40 (0.44)	16.02 (0.50)	15.62 (0.60)	13.44 (0.41)	14.46 (0.35)	14.36 (0.51)	-
Fruit traits (n = 3 fruits)							
Average seed number per fruit	45.67 (1.86)	48.33 (0.88)	45.67 (0.67)	39.33 (1.33)	59.33 (1.33)	54.67 (1.76)	-
Length (cm)	20.73 (0.33)	25.9 (4.16)	24.57 (0.84)	20.9 (0.93)	22.97 (1.08)	23.70 (0.32)	-
Width (cm)	9.60 (0.44)	10.3 (0.46)	9.07 (0.09)	8.43 (0.27)	9.97 (0.46)	7.73 (0.32)	-
Weight (g)	786.6 (68.8)	955.1 (133.8)	718.8 (27.4)	560.5 (48.8)	880.1 (110.2)	663.5 (39.9)	-
Pod husk (cm)	1.57 (0.27)	1.6 (0.15)	1.47 (0.09)	1.90 (0.06)	1.93 (0.09)	1.43 (0.12)	-
Pod husk (g)	562.3 (71.6)	668.7 (99.1)	515.8 (24.4)	435.6 (35.9)	659.7 (84.2)	429.3 (45.4)	-
Cacao seed traits (n = 9 seeds of 3 fruits)							
Length (cm)	2.84 (0.04)	2.73 (0.04)	2.69 (0.03)	2.39 (0.03)	2.40 (0.05)	2.51 (0.04)	-
Width (cm)	1.58 (0.02)	1.67 (0.03)	1.63 (0.02)	1.38 (0.02)	1.27 (0.02)	1.31 (0.02)	-
Depth (cm)	1.10 (0.03)	1.14 (0.03)	1.15 (0.02)	1.07 (0.03)	0.95 (0.02)	1.00 (0.01)	-
Weight (g)	3.04 (0.06)	3.28 (0.06)	3.24 (0.05)	2.39 (0.03)	1.80 (0.06)	1.98 (0.04)	-

Treatment 3: Inoculation with yeast and acetic acid bacteria

Inoculation liquids of yeast and acetic acid bacteria were produced on site to facilitate the adoption of this approach by farmers and to promote the development of the native regional microbial flora (see KOSTINEK et al., 2008 for lactic acid bacteria). To obtain yeast inoculation liquid, mature banana leaves were washed with well water, and the liquid was collected. Brown cane sugar and fresh lemon juice were added to yield a Brix value of 14–16% as measured with a refractometer (HI 96801, Hanna Instruments, Vöhringen, Germany) and a pH of 4–4.5 based on measurement with a HI98103 Checker device (Hannah Instruments, Vöhringen, Germany). To achieve anaerobic conditions, flasks were sealed with Parafilm and protected with aluminum foil against light.

For the production of the acetic acid bacteria inoculum, the same liquid from washed banana leaves with a Brix value of 13–14% and a pH of 4–4.5 was used. Then, 330 mL of the solution was mixed with 30 mL of 96% ethanol, 15 mL mother of vinegar (Vina Reinzucht Essigmutter, Schlag GmbH, Germany), and wood chips as medium. The mixture was kept in an open plastic bowl to achieve aerobic conditions. The inoculation liquids of both yeast and acetic acid bacteria were stored for 4 days at ambient temperature. The acetic acid liquid was sieved through 2 mm mesh to filter out wood chips. Afterwards 400 mL of the yeast inoculum and 200 mL of the acetic acid bacteria inoculum were mixed thoroughly with the cacao mass at the beginning of the fermentation.

Treatment 4: Control

For the control treatment, cacao seeds with pulp were put directly into the box without any additional treatment.

Pods for the pre-storage treatments were harvested on Mondays, for the other treatments on Thursdays. All fermentations were started simultaneously on Friday morning. For the pre-storage treatment, cacao was supplied in pods, whereas for the other treatments cacao pods were broken at the farms and seeds with pulp were placed in plastic drums sorted by clone. Prior to filling the boxes, a clone mixture was prepared according to Table 3, and the interior walls of the first box of each row were covered with banana leaves to support inoculation. Seeds from approximately 2,400 pods were placed in each of the four first boxes at the fermentation site. All fermentation masses were covered with banana leaves, jute bags, and a wooden lid. During the course of fermentation, the masses were olfactorily observed for the formation of ethanol and acetic acid. Furthermore, temperature was measured with a thermometer. In accordance with the observations the masses were transferred to the subsequent box (mixing) when ready for aeration (the timing was based on preliminary experiments). Depending on the mass conditions, two to three mixing events were undertaken during each fermentation.

The end of each fermentation was determined based on the color formation of the seeds and on the decreasing temperature of the fermentation mass. Subsequently, three buckets of 4 kg each, one from the upper, one from the middle, and one from the lower part of the last box, were separated for later analysis and sun-dried on marked jute bags in either of the two drying areas. The rest of the masses were sun-dried on white tarpaulins until they reached a residual water content of 6–8%.

Figure 15: **a** Fermentation site of the cooperative ASPROC-NBT; **b** drying facility with roof and sliding trays; **c** pre-drying of clone-wise sorted pods; **d** recently entered cacao mass with thermometer for temperature measurement.

3.3.3 On-site analysis

Prior to fermentation, the pulp volume of each clone was measured according to PASS (1996) by taking at least 30 seeds with pulp from three different fruits. For the pod storage treatment, the procedure was conducted before and after sun drying of the pods. The Brix value was measured with a refractometer (HI 96801, Hanna Instruments, Vöhringen, Germany). The pulp of eight seeds was drained into a plastic cup, and the liquid transferred to the refractometer. Temperature was measured twice a day with a fermentation thermometer (Bitherma, Kl. 1, Lemgo, Germany, total length 60 cm) in all fermentation boxes at three locations per box above the center of the box's base area: in the upper (10 cm depth), middle (30 cm depth) and bottom parts (50 cm depth) (Fig. 15d).

Mean overall box temperature was calculated as the mean of the three measurements. The pH value of the pulp was measured once a day from two samples in each box: three cacao seeds were taken from a depth of approximately 15 cm of the mass and shaken in 20 mL of purified water in a 50 mL Falcon tube for 15 s (HI98103 Checker, Hannah Instruments, Vöhringen, Germany). In addition, the odor of the masses was recorded twice a day, with the categories alcohol, acetic acid, bad odor, and other odors, each on a scale from 1 (little) to 3 (strong). Fermentation quality was assessed with the cut test: 100 dried raw cocoa beans were cut longitudinally and examined for quality (DEL BOCA, 1962; GUEHI et al., 2010).

3.3.4 Sensory analysis of cocoa liquor

After fermentation and drying, a representative sample of 1 kg raw cocoa beans was taken from each fermentation batch. Raw cocoa beans were processed according to a standard protocol of APPCACAO Peru (Asociación Peruana de Productores de Cacao) by roasting and subsequent production of cocoa liquor. Panelists of APPCACAO Peru degusted the cocoa liquor samples according to standard quality criteria on a scale from 1 to 10 (1 = little taste/flavor, 10 = very strong taste/flavor) using the common classifications: cacao, acidity, astringency, bitterness, fruity, floral, nutty, raw, earthy, chocolate, as well as off-flavors such as smoky and moldy taste (compare SUKHA et al., 2008).

3.3.5 Laboratory analyses

3.3.5.1 Chemicals and standards

All chemicals used were of analytical grade and, unless otherwise specified, were obtained from Merck (Darmstadt, Germany). Epicatechin, catechin hydrate, cyanidin-3-arabinoside, cyanidin-3-galactoside, and amino acids as standard for HPLC analyses were provided by Sigma/Fluka (Sigma-Aldrich Chemie/Fluka, Taufkirchen, Germany). Deionized water was produced using an Elga water purification system (PURELAB Option, Elga, UK).

3.3.5.2 Degreasing of cacao seeds

Aliquots of fermented and dried seed samples for each experimental run were stored in plastic bags and transferred to the lab in Hamburg. Each sample was freeze-dried for 72 h (SciQuip Ltd., Christ Alpha I-6, Newtown, UK). Subsequently, seed shells were removed. About 2 g of each cotyledon sample were defatted through milling to a particle size of approx. 1 μm^3 in a ball mill (MM200, Retsch, Haan, Germany) at a frequency of 20 s^{-1} for 10 min after adding 10 mL *n*-hexane. The grist was washed three times with 25 mL petroleum ether (boiling range 40–60 °C) with a 0.45 µm filter in

a Büchner funnel. The fat-free filter cake was vacuum-dried at ambient temperature for 1 h at 100 mbar using a vacuum oven (Hereaus, Hanau, Germany).

3.3.5.3 Extraction and chromatographic analysis of amino acids

Free amino acids were extracted and analyzed according to the method described by ROHSIUS et al. (2006) with slight modifications: 0.1 g defatted cocoa powder was mixed with 0.3 g polyvinyl-polypyrrolidon (PVPP) to remove polyphenols and 10 mL of deionized H_2O. The pH value was adjusted to pH 2.5 with 50% aqueous trifluoroacetic acid solution and the solution stirred at <4 °C for 1 h. Samples were centrifuged for 10 min at 4100 rpm (Thermo Scientific, Mega-Fuge 11 R Centrifuge, Heraeus, Hanau, Germany). The clear supernatant was filtered through a 0.45 µm syringe filter, and 30 µL of each sample was lyophilized (1 h; −20 °C, 0.05 mbar). The lyophilized samples were kept at −20 °C until analysis. Further sample preparation and determination of the concentrations of free amino acids with RP-HPLC were conducted according to ROHSIUS et al. (2006). Samples were measured against calibration series of the respective amino acids at λ_{ex} = 334 nm and λ_{em} = 425 nm. Concentration of amino acids was tested separately for each amino acid and grouped according to the chemical properties of amino acids (all, acidic, hydrophobic, and other amino acids).

3.3.5.4 Extraction and chromatographic analysis of organic acids

Sample preparation and the analysis of lactic and acetic acid with RP-HPLC were performed according to a method adapted from ROHSIUS (2008). First, 2 g of degreased cocoa powder was mixed with 10 mL of 0.2% benzoic acid solution and extracted in a ball mill at a frequency of 25 s^{-1} for 10 min. The homogenate was centrifuged for 10 min at 4100 rpm and the resulting supernatant centrifuged again in an Eppendorf tube at 13,000 rpm for 10 min (Biofuge fresco, Heraeus, Hanau, Germany). The supernatant was filtered through a 0.45 µm syringe filter, and an aliquot of 1 mL was applied to freshly conditioned BAKERBOND® anion exchange cartridges. Conditioning of cartridges, cleaning of the extract, RP-HPLC conditions, and the determination of lactic and acetic acid at λ = 215 nm against calibration series of the respective acids were carried out according to ROHSIUS (2008).

3.3.5.6 Extraction and analysis of total polyphenols

The content of total polyphenols was determined with the Folin-Ciocalteu method described by SINGLETON AND ROSSI (1965). The extraction procedure and analysis of the total polyphenol concentration with a spectrophotometer (Uvikon 943, Double Beam UV/VIS Spektrophotometer, Kontron Instruments, Rossdorf, Germany) were conducted according to KIECK et al. (2016).

3.3.5.7 Extraction and chromatographic analysis of polyphenolic compounds

Sample preparation and measurement of polyphenolic compounds by RP-HPLC were conducted according to ELWERS et al. (2009) with moderate modifications. To start, 5 mL of methanol was added to 0.1 g of defatted cocoa powder, the mixture stirred for 20–30 s with an ULTRA-TURRAX T25

agitator (Janke & Kunkel, Staufen, Germany), and then rinsed with 2 mL methanol. The solution was placed in an ultrasonic bath (Sonorex, Super RK 510 H, Bandelin, Berlin, Germany) for 3 min, subsequently cooled on ice for 15 min, and centrifuged at 4100 rpm for 10 min. This extraction procedure was repeated twice, and the three supernatants were collected. The methanol of the united extracts was withdrawn using a rotary evaporator (LABO Rota SE 320, Resona Technics, Gossau, Switzerland; 40 °C, 100 mbar). Afterwards, the residue was dissolved in 3 mL methanol and filtered (syringe filter, 0.45 µm). The determination of the phenolic compound concentration was conducted by RP-HPLC following ELWERS et al. (2009) and NIEMENAK et al. (2006). Phenolic compounds were detected against a calibration series of the respective polyphenols at λ = 280 nm. Total polyphenols based on HPLC were calculated based on the sum of all compounds detected.

3.3.6 Statistics

All statistical tests were carried out with R, version 3.1.1 (R DEVELOPMENT CORE TEAM, 2014). Differences among treatments concerning temperature development and contents of organic acids, amino acids, and polyphenols were assessed using repeated measures of analysis of variance (ANOVA) with "treatments" nested in "experiment weeks" (QUINN AND KEOUGH, 2002). This was followed by a Tukey HSD post-hoc test whenever differences among treatments were significant. In addition, linear regression models were calculated for predicting contents of organic acids, amino acids, and polyphenols in dried raw cocoa beans (CRAWLEY, 2012). Only predictor variables with a Pearson-Bravais correlation coefficient <0.5 were included in a model to prevent multicollinearity (CRAWLEY, 2012).

Odor development and flavor traits were assessed on an ordinal scale and hence did not meet the criterion of Gaussian distribution. Therefore, these data were analyzed based on non-parametric techniques (CRAWLEY, 2012). Differences among treatments were tested by Kruskal-Wallis tests, followed by pairwise Mann-Whitney U-tests in case of significant differences. Correlations of flavor traits with temperature regime, organic acids, amino acids, and polyphenols were assessed using Spearman's rank correlation rho (CRAWLEY, 2012).

To reduce the dimensionality of the sensory profile of the cocoa liquor, the sensory characteristic matrix was analyzed by using correspondence analysis (CA; NENADIĆ AND GREENACRE, 2007). The first two dimensions explained 52.4% and 28.6% of the overall variation in the sensory profile and were used as condensed measurements of the sensory quality of the cocoa bean samples.

Table 4: Means (standard error) of temperature and contents of free amino acids, polyphenols, and organic acids related to fermentation treatments.

	Pod storage	**Pulp removal**	**Inoculation**	**Control**
Temperature				
Maximum overall box temperature (°C)	45.8 (0.9)	44.7 (1.4)	47.4 (0.1)	46.2 (0.4)
Temp. rise (0-48 h of fermentation; °C h^{-1})	0.099 (0.024)	0.155 (0.011)	0.148 (0.006)	0.140 (0.012)
Temp. rise (0-144 h of fermentation; °C h^{-1})	0.098 (0.015)	0.104 (0.016)	0.125 (0.010)	0.116 (0.008)
Temp. rise (15-144 h of fermentation; °C h^{-1})	0.101 (0.020)	0.092 (0.017)	0.120 (0.017)	0.106 (0.013)
Fermentation quality (% well-fermented beans based on cut test)	47.0 (6.9)	50.6 (5.7)	52.5 (3.6)	55.1 (2.9)
Content of free amino acid in raw cocoa beans (mg/g fat-free dry matter)				
Total amino acids	19.07 (1.87)	19.00 (1.97)	16.39 (0.48)	16.93 (1.16)
Hydrophobic amino acids	8.85 (0.86)	9.18 (1.04)	7.63 (0.24)	7.98 (0.66)
Acidic amino acids	3.63 (0.28)	3.59 (0.29)	3.02 (0.15)	3.09 (0.26)
Other amino acids	5.30 (0.72)	5.19 (0.52)	4.80 (0.11)	4.93 (0.26)
Alanine	1.58 (0.16)	1.82 (0.20)	1.47 (0.05)	1.50 (0.10)
Arginine	1.29 (0.17)	1.44 (0.16)	1.25 (0.04)	1.32 (0.08)
Asparagine	1.55 (0.10)	1.45 (0.14)	1.17 (0.08)	1.21 (0.10)
Aspartic acid	0.39 (0.03)	0.37 (0.03)	0.33 (0.02)	0.32 (0.02)
GABA	1.29 (0.13)	1.03 (0.16)	0.94 (0.07)	0.92 (0.10)
Glutamine	0.17 (0.03)	0.18 (0.01)	0.16 (0.02)	0.17 (0.02)
Glutamic acid	1.53 (0.15)	1.59 (0.12)	1.36 (0.07)	1.40 (0.13)
Glycine	0.31 (0.05)	0.31 (0.05)	0.26 (0.01)	0.27 (0.00)
Isoleucine	0.69 (0.07)	0.68 (0.08)	0.58 (0.02)	0.60 (0.05)
Leucine	2.40 (0.23)	2.47 (0.28)	2.06 (0.05)	2.18 (0.17)
Lysine	1.67 (0.20)	1.63 (0.22)	1.44 (0.06)	1.60 (0.13)
Methionine	0.25 (0.06)	0.23 (0.08)	0.00 (0.00)	0.09 (0.09)
Phenylalanine	2.02 (0.20)	2.07 (0.27)	1.67 (0.06)	1.78 (0.17)
Serine	0.63 (0.06)	0.69 (0.08)	0.57 (0.01)	0.60 (0.04)
Threonine	0.43 (0.06)	0.45 (0.06)	0.38 (0.02)	0.39 (0.04)
Tryptophan	0.71 (0.26)	0.44 (0.26)	0.89 (0.03)	0.67 (0.23)
Tyrosine	1.22 (0.11)	1.19 (0.11)	1.03 (0.03)	1.09 (0.08)
Valine	0.94 (0.10)	0.95 (0.11)	0.81 (0.05)	0.83 (0.09)

Content of polyphenols in raw cocoa beans (mg/g dry mass)				
Total polyphenols (Folin-Ciocalteu)	71.69 (9.78)	59.37 (6.60)	58.17 (3.78)	59.53 (3.51)
Total polyphenols (HPLC)	5.60 (1.83)	2.35 (0.68)	2.53 (0.55)	2.41 (0.44)
Epicatechin	5.23 (1.68)	2.26 (0.62)	2.41 (0.53)	2.38 (0.40)
Catechin	0.37 (0.16)	0.09 (0.07)	0.12 (0.05)	0.04 (0.04)
Content of organic acids in raw cocoa beans (mg/g fat-free dry matter)				
Acetic acid	10.23 (3.74)	10.60 (3.38)	10.77 (3.13)	9.93 (3.25)
Lactic acid	25.34 (1.40)	23.63 (1.38)	23.17 (0.94)	22.96 (0.96)

3.4 Results

3.4.1 Impact of pre-conditioning treatments on the temperature development during fermentation

During cacao fermentation in general, the temperature changes according to microbial activity in the fermentation mass. The temperature development in these experimental fermentations followed the expected patterns of a typical cacao fermentation (Fig. 16). The results revealed a significant relation between the temperature increase within the first 48h and the treatment (Fig. 17a). Significantly faster temperature increase in the boxes with pulp drainage were found compared to the boxes with pre-stored pods. In the natural and inoculation treatments, the temperature increase was intermediate and did not significantly differ from the other treatments. During other periods of the fermentation process, no further differences in temperature were measured.

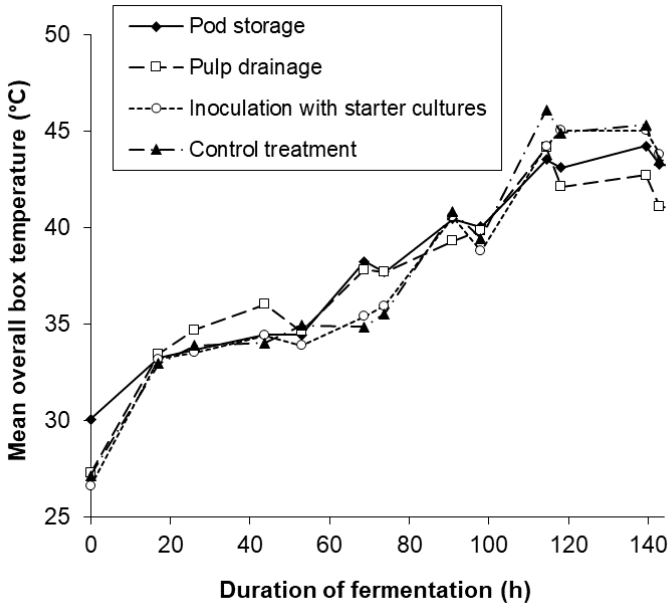

Figure 16: Temperature development (mean box temperature of four consecutive fermentations) during fermentation after different pulp pre-conditioning treatments.

These findings were supported by the odor development of acetic acid during the fermentation (Kruskal-Wallis rank sum test, n = 16, chi-squared = 11.868; p = 0.0078). Significantly less acetic acid odor was perceived in the boxes with the pre-stored pods and in the inoculated boxes compared to the boxes treated with pulp removal and to the natural treatment (pairwise Mann-Whitney U-tests). However, the analysis of acetic acid formation after 6 days of fermentation, which corresponded to the end of the process, did not reveal significant differences among the treatments. The same was true for lactic acid after 6 days.

The cut test of fermented beans showed no significant effect of treatments, but a significant positive correlation of cut test with the maximum overall box temperature was found (Fig. 17b).

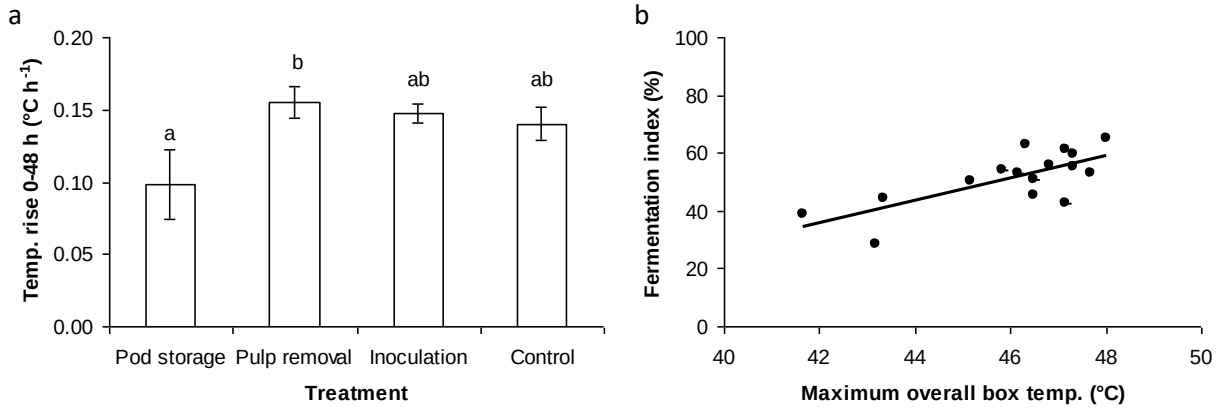

Figure 17: Effect of **a** pre-conditioning treatment on temperature increase during the first 48 h of fermentation (ANOVA, n = 16, F = 4.55; p = 0.0333; different lowercase letters indicate significant differences according to Tukey HSD test) and of **b** maximum overall box temperature on fermentation quality according to the cut test (visual assessment of 100 longitudinally cut raw cocoa beans; n = 16, r^2 = 0.531, p = 0.0014).

3.4.2 Impact of treatments on free amino acids and polyphenols in raw cocoa beans

The results indicate that there was little or no difference in most of the amino acids and polyphenols after the fermentation process. In an exception, it was found that the release of free asparagine during fermentation differed significantly across the treatments with the pre-stored pods showing significantly higher content compared with the inoculation and natural treatments, while the cacao beans with pulp removal showed intermediate results (Fig. 18a). Methionine was significantly more concentrated after applying the pre-storage treatment compared to inoculation (ANOVA, n = 16, F = 4.75, p = 0.0299; Tukey HSD post-hoc test: p = 0.0411). Accordingly, the total content of acidic amino acids (asparagine, aspartic acid, glutamine, and glutamic acid) of the pre-stored pods was marginally significantly higher than the inoculated beans (ANOVA, n = 16, F = 4.22; p = 0.0403, Tukey HSD post-hoc test: p = 0.0873). Fermentation temperature proved to be the most important driver of free amino acid formation with higher maximum overall box temperature leading to lower amino acid

content (total amino acids: Fig. 18b; hydrophobic amino acids: n = 16, r^2 = 0.456, p = 0.0041; acidic amino acids: n = 16, r^2 = 0.471, p = 0.0034; other amino acids: n = 16, r^2 = 0.505, p = 0.0020). With the exception of three amino acids (aspartic acid, lysine, and tryptophan), all amino acids showed a significantly negative correlation with maximum overall box temperature (data not shown).

Although differences in total polyphenols were not found, epicatechin concentration in the beans was marginally significantly higher in beans fermented from the pre-stored pods compared to the beans with pulp removal (ANOVA, n = 16, F = 3.71, p = 0.0549, Tukey HSD post-hoc test: p = 0.0804) and the control (Tukey HSD post-hoc test: p = 0.0946). Again, the temperature significantly influenced the content of polyphenols with the temperature increase during the first 48 h of fermentation consistently showing a negative correlation with polyphenol content (total polyphenols according to Folin-Ciocalteu method: n = 16, r^2 = 0.430, p = 0.0058; total polyphenols according to HPLC: n = 16, r^2 = 0.379, p = 0.0112; epicatechin: n = 16, r^2 = 0.365, p = 0.0132, catechin: n = 16, r^2 = 0.472, p = 0.0033). The inclusion of lactic acid content in dried raw cocoa beans into the models additionally revealed significant positive correlations of lactic acid with total polyphenols based on HPLC [p = 0.0037, multiple r^2 = 0.682, p(model) = 0.0006], epicatechin [p = 0.0033, multiple r^2 = 0.682, p(model) = 0.0006], and catechin [p = 0.0398, multiple r^2 = 0.623, p(model) = 0.0018].

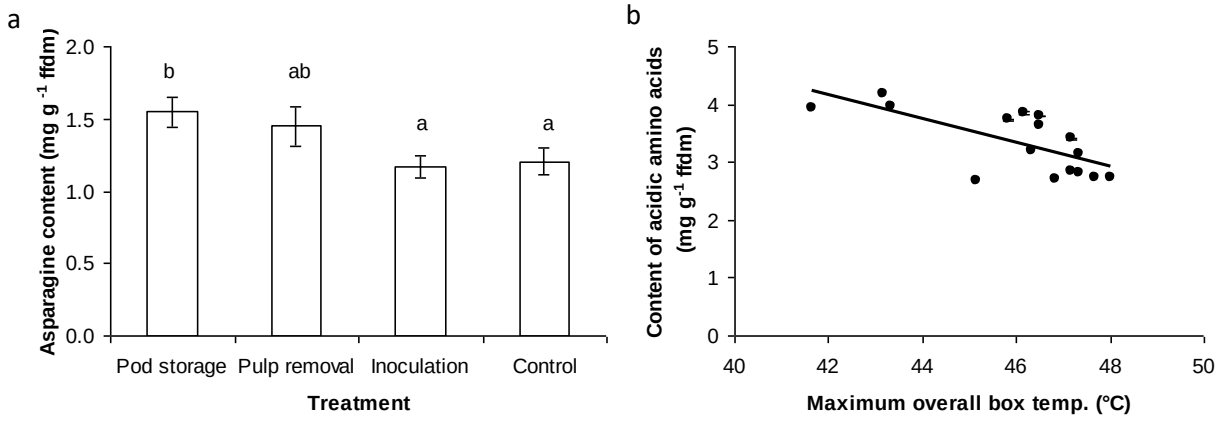

Figure 18: Effect of **a** fermentation treatment on the concentration on free asparagine in raw cocoa beans (ANOVA, n = 16, F = 8.17, p = 0.0062; different lowercase letters indicate significant differences according to Tukey HSD test) and of **b** maximum box temperature on the concentration of total free acidic amino acids in raw cocoa beans (n = 16, r^2 = 0.524, p = 0.0015). ffdm = fat-free dry matter.

3.4.3 Impact of treatments on sensory characteristics of cocoa liquor

There was no evidence that individual sensory characteristics or the overall sensory profile of the fermented raw cocoa beans was directly influenced by the fermentation treatments. However, the sensory characteristic “cacao” was significantly more pronounced when the post-fermentation content of free acidic amino acids was higher (n = 16, rho = 0.515, p = 0.0411). Moreover, significant positive correlations of the “cacao” flavor characteristic with the amino acids alanine (n = 16, rho =

0.569, p = 0.0213), asparagine (n = 16, rho = 0.542, p = 0.0300), and valine (n = 16, rho = 0.542, p = 0.0300) were found. In contrast, the sensory characteristic “acidic” was negatively correlated with the temperature increase during the first 144 h of fermentation (n = 16, rho = -0.521, p = 0.0383) and positively correlated with the total concentration of polyphenols as determined by the Folin-Ciocalteu method (n = 16, rho = 0.631, p = 0.0088). Astringent flavor characteristics were marginally negatively related to the temperature increase during the first 144 h of fermentation (n = 16, rho = -0.444, p = 0.0846) and showed a negative correlation with the final content of lactic acid in cocoa beans (n = 16, rho = -0.582, p = 0.0180). The sensory characteristic “bitter” exhibited a significant negative correlation with the temperature increase during the first 48 h of fermentation (Fig. 19a), whereas the characteristic “nutty” was positively correlated with the temperature increase during the first 48 h (Fig. 19b). These results were also reflected in a negative temperature response (temperature increase between hours 15 and 144 of fermentation: n = 16, r^2 = 0.262, p = 0.0425) of the second dimension of the CA, which had a positive correlation with astringent and bitter notes and a negative correlation with chocolate notes. Overall, these findings point to an impact of temperature change during the fermentation process on the sensory profile with lower temperatures leading to more acidic, astringent, and bitter characteristics. Furthermore, the content of acidic amino acids had a positive effect on a typical “cocoa” aroma. Interestingly, there were no direct effects of treatment, temperature, or content of amino acids and polyphenols on the characteristics “floral” and “fruity” in the experiment.

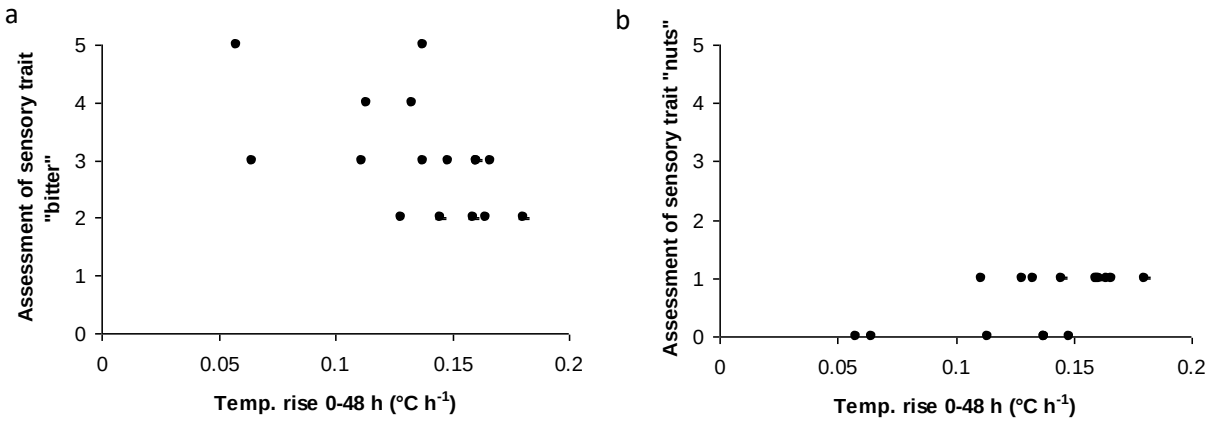

Figure 19: Effect of the temperature increase during the first 48 h of fermentation on **a** bitter (n = 16, rho = -0.516, p = 0.0409) and **b** nutty (n = 16, rho = 0.532, p = 0.0339) aroma notes in cocoa liquor.

3.5 Discussion

This study provides novel insights into the potential for pulp pre-conditioning techniques to optimize temperature development and resulting biochemical and flavor characteristics of high-quality cacao clones. It is one of the first studies to explicitly address South American fine and flavor cococa (but see TROGNITZ et al., 2013) and to compare different pre-conditioning methods (see PASS, 1996).

3.5.1 Impact of treatments on temperature development during fermentation

Temperature development during fermentation indicates successful fermentation in all treatments with similar maximum overall box temperature and final fermentation quality as determined by the cut test. Also, a fermentation duration of 120–144 h is typical for the plant material used (SALTINI et al., 2013). As expected from studies in Asia (MEYER et al., 1998; PASS, 1996), pod storage resulted in significantly smaller temperature increase during the first 48 h of fermentation. However, despite significantly lower acetic acid odor during fermentation, there was no evidence of a decrease in acidity in the raw cocoa beans after pod storage, which was the objective of the experiments in Asia. In this study, the smaller temperature increase at the beginning of fermentation (as a consequence of pod storage) caused a clear change in the concentrations of polyphenols and sensory characteristics (see below) and may therefore be used to modify cocoa bean quality. Accordingly, AFOAKWA et al. (2013) found extensive changes in chemical composition and physical characteristics of cocoa beans after pod storage with apparently beneficial effects on cocoa quality. In the experiment, moisture reduction owing to pod storage ranged from 20% to 48% in the clones used (Tab. 3). Consequently, the effect of pod storage may be further fine-tuned by varying the percentage of each clone in the fermentation mass.

In contrast to pod storage, mechanical pulp removal led to a significantly greater temperature increase at the beginning of the fermentation, and fermentation time was shorter than in the other treatments (Fig. 16). The techniques of pre-drying and pulp drainage were originally undertaken to reduce yeast development at the beginning of the fermentation and the final acidity of the beans (BIEL et al., 1990). In this study, pulp drainage caused the opposite trend. This surprising result may be attributed to the mechanical squeezing applied in this experimental set-up, which reduced the water content of the mass, possibly facilitating faster warming without corroborating yeast development. The reduced amount of substrate, on the other hand, may have caused the earlier ending of the fermentation process. In keeping with this, acceleration of fermentation through pulp removal was described by SCHWAN AND WHEALS (2004) for Brazilian cacao, but the fermentation time did depend on the depulping methods (mechanical, enzymatic, washing). In the treatments, maximum overall box temperature was comparable but slightly lower after pulp removal than in the control, which indicates some effect of treatment although it was not significant.

Inoculation with starter culture preparation showed no effect on fermentation. A significantly less pronounced acetic acid odor during fermentation points to reduced activity of acetic acid bacteria, although these were added at the beginning of the fermentation. Similarly, KRESNOWATI AND FEBRIAMI (2016) found poor fermentation after using *Acetobacter* species as starter culture. However, in terms of the temperature development, no evidence was found that the prepared inocula can be used to influence the fermentation process. Still, specific microorganisms within the fermentation mass are

known to cause accelerated fermentation (SCHWAN AND WHEALS, 2004; KRESNOWATI et al., 2013; KRESNOWATI AND FEBRIAMI, 2016), improved and more stable bean quality (LEFEBER et al., 2010; KONÉ et al., 2016), or may counteract the growth of undesired or even pathogenic microorganisms in the fermentation mass (SALTINI et al., 2013; MAHAZAR et al., 2015). The use of more specific inocula may therefore be recommended, if this does not lead to pronounced added costs for the farmers, which was explicitly aimed to avoid through this approach.

Overall, pod storage represents a pulp pre-conditioning technique with smaller temperature increase at the beginning of the fermentation, whereas pulp removal may be used to accelerate fermentation in this clone mixture. Fermentation success depended on the maximum temperature reached during fermentation rather than initial temperature increase, as indicated by the positive correlation between the cut test and the maximum overall box temperature. Therefore, the treatments represent a way of modifying temperature without disturbing the desired fermentation process (see BIEHL et al., 1990).

3.5.2 Impact of treatments on free amino acids and polyphenols in raw cocoa beans

Along with the temperature, pod storage also influenced the formation of free amino acids with significantly higher concentration of asparagine and methionine and with marginally higher content of acidic amino acids in the dried cocoa beans compared to other treatments. In accordance, HANSEN et al. (2000) found higher activity of proteases after pod storage, which results in enhanced cleavage of storage proteins and amino acid liberation. As amino acids are the relevant aroma precursors that ultimately produce the chocolate aroma in the Maillard reaction during roasting (e.g., APROTOSOAIE et al., 2016), these findings make pod storage a potentially effective method to adapt the aroma profile of Peruvian fine and flavor cacaos to the consumers' demand. This is supported by a significant correlation of acidic amino acids and asparagine with the flavor characteristic "cacao." Interestingly, a higher maximum overall box temperature led to significantly lower content of amino acids in raw cocoa beans in the clone mixture used. Although acidic amino acids (and in particular asparagine) are known to be released prior to fermentation and may therefore show kinetics that differ from other amino acids (KIRCHHOFF et al., 1989b), which may be the reason for their response to fermentation treatment, these data imply that overall protease activity (aspartic endoproteases and carboxypetidases; see HANSEN et al., 2000, AFOAKWA et al., 2008) is positively influenced by lower maximum temperature. The increased liberation of specific amino acids indicates optimum conditions for specific proteases at different fermentation regimes. Accordingly, HANSEN at el. (2000) show clear changes in enzymatic activity of different enzyme classes with pod storage. However, the

question of whether these treatments indeed influence the proteomics of the cleavage process requires further experimental evidence.

In terms of polyphenols, pod storage again differed from the other treatments with a marginally significantly higher concentration of epicatechin in pre-stored beans. In addition, temperature increase during the first 48 h of fermentation, which was lower in pre-stored pods compared to those undergoing pulp removal, proved to be significantly negatively correlated to the final polyphenol content. This is in line with the expectation, as polyphenol content in raw cocoa beans depends on the temperature development during fermentation (e.g., TOMAS-BARBERÁN et al., 2007), and polyphenol loss is most pronounced during the first 48 h of fermentation (ALBERTINI et al., 2015). However, AFOAKWA et al. (2012a) found significantly lower polyphenol contents after pod storage, which may be attributed to much longer storage time in their experiments (up to 21 days). Further analyses based on shorter pod storage times accordingly revealed increased polyphenol contents after 3 days compared to no pod storage despite an overall negative correlation of storage time and polyphenol content in African clones (AFOAKWA et al., 2015).

The results also point to a certain relevance of the microaerobic phase: the positive correlation between the contents of lactic acid and polyphenols shows that an early start of vigorous lactic acid bacteria activity during fermentation impedes polyphenol decomposition. This highlights the role of lactic acid bacteria for aroma production in Peruvian fine and flavor cacao clones, as already proposed for Nigerian fermentations by KOSTINEK et al. (2008). Both the content of polyphenols and the final raw cocoa bean pH have clear consequences for sugar and free amino acid formation and thus for the flavor characteristics of the final chocolate (NOOR-SOFFALINA et al., 2009). Again, pod storage and pulp removal represent valuable methods for modifying polyphenol content according to consumers' wishes with higher content after pod storage compared to pulp removal.

3.5.3 Impact of treatments on sensory characteristics of cocoa liquor

Sensory characteristics of the clone mixture used showed no significant direct relation to the pulp pre-conditioning treatments applied. However, significant links were found to the concentrations of free amino acids and polyphenols along with the temperature regime, all of which were influenced by the fermentation treatment. The flavor note "cacao" was positively related to free amino acids (alanine, asparagine, valine, and acidic amino acids). In particular alanine and valine are known to be main precursors of the typical chocolate aroma (NOOR-SOFFALINA et al., 2009). Acidic flavor positively responded to the content of polyphenols, and astringent flavor was positively correlated with lactic acid. Similar relations of flavor characteristics with chemical compounds were recently published by TROGNITZ et al. (2013) for fine and flavor cacaos grown in Nicaragua. In contrast to these results, the

authors found the expected significantly positive relation of epicatechin with astringent and bitter flavors. In this study, the polyphenols were positively correlated with lactic acid, a compound that similarly reduces the flavor quality of cacao (RODRIGUEZ-CAMPOS et al., 2011). The observed relationship of astringency and lactic acid seems to indicate the effect of polyphenols on the flavor profile, even though the direct correlation of polyphenols and astringency was not significant.

Table 5: Sensory characteristics (median) and correspondence analysis scores of sensory profiles (mean, standard error).

	Pod storage	**Pulp removal**	**Inoculation**	**Control**
Sensory characteristic				
Cacao	2.5	3	2.5	2
Acid	3	2	3	2
Astringency	3	3	2.5	2.5
Bitter	3	3	2.5	2.5
Fruity	2	2	1.5	1.5
Floral	1.5	1	1	1.5
Nutty	0.5	0.5	1	1
Raw	0	0	0	0
Chocolate	2	0.5	0.5	1.5
Sensory profile				
CA score of the 1st dimension	3.54 (0.32)	3.47 (0.25)	3.61 (0.27)	3.00 (1.00)
CA score of the 2nd dimension	2.11 (0.63)	2.20 (0.48)	1.99 (0.52)	0.95 (0.34)

Moreover, significantly negatively correlated effects of temperature increase on acidic, astringent, and bitter notes were found, which were also reflected in the overall aroma profile. In contrast, the flavor characteristic “nutty” was positively influenced by greater increase in temperature at the beginning of the fermentation. Overall, these results show that the flavor profile may be indirectly changed through the pre-conditioning treatments with more pronounced acidity, astringency, and bitterness after pod storage compared to pulp removal. Typical notes of fine and flavor cacaos such as floral or fruity showed no relation to amino acids, polyphenols, or temperature development. These aroma notes are already present in the fruit pulp (terpenoids or esters; KADOW et al., 2013) and do not derive from microbial activity during fermentation. This may be the reason why these aroma traits show no relation with fermentation treatment and rather are dependent on the clones used.

3.6 Conclusion

The study shows that pulp pre-conditioning techniques are valuable methods to influence the fermentation process, the content of aroma-relevant compounds, and the flavor profile of raw cocoa beans in fine and flavor cacaos of Peru. Pod storage resulted in a smaller temperature increase and higher contents of free amino acids and polyphenols. In contrast, pulp removal—when carried out smoothly as in this experiment—led to a more pronounced temperature increase at the beginning of the fermentation along with lower contents of amino acids and polyphenols. The changes in the biochemical composition of cocoa beans were also reflected in the resulting aroma profile. Consequently, the treatments may be used to modify the aroma profile of the clones used in Peru.

Farming conditions (soil, field biodiversity) are also known to affect the initial contents of polyphenols and methylxanthines in cacao seeds (KIECK et al., 2016). These conditions should be considered in future fermentation experiments. Overall, the results can support farmers to produce raw cocoa beans of defined quality and to better meet differing market demands.

4 FERMENTING IN A BOX: DO CACAO CLONE AND VESSEL WOOD TYPE MATTER?

4.1 Abstract

Cocoa is one of the world's most important commodity cash crops, with quality aspects becoming increasingly important. Therefore, apart from breeding novel cacao varieties, adequate post-harvest treatments are explored in all major cacao producing regions of the world. In particular, well-designed fermentation techniques are crucial for obtaining a high-quality product with special flavor characteristics. However, little is known about the requirements for the fermentation of different cacao clones in a fermentation mass and about the impact of fermentation vessel material on the process. This study explored the differences of eight Trinitario and one Forastero clone cultivated in Peruvian Amazonia regarding several parameters characterizing the fermentation process. The analyzes were based on 49 single clone micro-fermentations in 27 L boxes made of different types of wood, with and without polystyrene insulation. As wood type seemed to play a role during these fermentations, additional 41 fermentations with clone-mixtures in wooden boxes made of four typical tree species were executed. In all fermentations, temperature development, fermentation degree of raw cocoa beans and content of amino acids were analyzed. The study was carried out in Tocache, San Martín, Peru using defined Trinitario clones (ICS-1, ICS-6, ICS-39, ICS-60, ICS-95, IMC-67, TSH-565, UF-29 and UF-613). This study revealed significant differences in the temperature development during fermentation between clones and fermentation vessel materials. Temperature increase within the first 48h of ICS-95 was significantly different to IMC-67 and UF-613. Insulation of the boxes caused a significantly less pronounced temperature increase between 48 and 96 h of fermentation—presumably due to reduced inoculation and aeration of the fermentation mass. After 96 h, however, cooling of the mass was significantly slower. Additionally, the mixed-clone fermentations showed that temperature development and the formation of free amino acids were significantly influenced by the timber type of fermentation vessel. The findings provide evidence that different clones ferment at varying temperature profiles and that certain fermentation vessel materials are more appropriate for the process than others. Based on the findings, clones with similar fermentation properties may be processed together, which leads to a more uniform quality. Furthermore, adequate fermentation vessel material should be used to optimize cacao quality.

4.2 Introduction

World cocoa production is continuously growing (WORLD COCOA FOUNDATION, 2014) together with increasing consumer interest in fine and flavor cocoa (ICCO, 2016) and healthier chocolate products (SCHWAN AND WHEALS, 2004). Fine and flavor cocoa includes Criollo and Nacional cultivars as well as partially Trinitario varieties that are hybrids between Criollo and Forastero, the latter being of bulk cocoa quality (LIEBEREI AND REISDORFF, 2012). The classification of fine and flavor cocoas mainly relies on specific flavor attributes such as flowery, fruity, nutty or caramel notes in addition to a decent cacao aroma (LIEBEREI ET AL., 2010). These aroma notes are caused by volatile compounds inherent in cacao pulp that migrate into the cacao seed during fermentation (KADOW et al., 2013). Moreover, the aroma precursors (free amino acids, oligopeptides, and reducing sugars) are produced during fermentation and are necessary for the formation of cocoa and chocolate aroma via the Maillard reaction and Strecker degradation during the roasting and conching process (ROHSIUS, 2006). Consequently, the quality of the raw cocoa beans (fermented and dried seeds) depends on the genetic background (NIEMENAK et al., 2006; KONGOR et al., 2016), the farming conditions (ELWERS et al., 2006; KIECK et al., 2016), and also the post-harvest treatments–fermentation and drying (ROHSIUS, 2006). In bulk cocoa, fermentation aims exclusively at the production of the typical chocolate flavor, whereas fine and flavor cocoa fermentation also seeks to conserve the clone-specific volatile aroma components (APROTOSOAIE et al., 2016; KADOW et al., 2013). Bulk cocoa is fermented up to eight days; in contrast, fine and flavor cocoa ferments in much less time, with some Criollo cultivars taking 2 to 3 days only. Trinitario varieties commonly require a fermentation duration of 5 to 6 days. However, due to their heterogeneity, adequate conditions vary. Consequently, fermentation conditions are adjusted based on the genetic traits and environmental conditions, which refers to e.g., the duration, mixing intervals, and pre-treatments (e.g., GUEHI et al., 2010; SALTINI et al. 2013).

Since 2008, Peru has been classified by the International Cocoa Organization (ICCO) as a fine and flavor exporting country, with currently 75% export share (ICCO, 2016). In contrast to bulk cacao, fine and flavor varieties may yield a bonus above the commodity stock market price if they are well-processed and thus of good quality (ICCO, 2016). However, since the 1990s, the high yielding and highly resistant bulk cocoa clone CCN-51 has been cultivated mainly in the largest cacao growing area of Peru, in San Martín (GARCÍA CARRIÓN, 2012). To keep the fine and flavor status, as well as meeting consumer demands and ensuring genetic diversity and more stable incomes for farmers, Trinitario cacao has been planted in the region of San Martín since 2009 (GÓMEZ ALIAGA et al., 2014). Therefore, the propagation and cultivation of six international fine and flavor Trinitario clones has been promoted (ICS-1, ICS-6, ICS-39, ICS-95, TSH-565, and UF-613), together with one Forastero clone (IMC-67) for improved fruit set (GÓMEZ ALIAGA et al., 2014). The selection of the clones was based on

their productivity (2–3.5 t ha^{-1} $year^{-1}$ in well-maintained plantations with planting density of 1,111 individuals per ha), their pest resistance, and—most importantly—on their flavor and aroma characteristics. Trinitario clones are planted in mixed-clonal plantations in agroforestry systems (GÓMEZ ALIAGA et al., 2014). As these clones were re-introduced to Peru, respectively recovered from old cacao gene banks in Peru, experience with handling is lacking. Especially fermentation procedures adapted to Trinitario fine and flavor cocoa clones cultivated in Peru are not available yet. This may affect the marketing of the final product. A poorer overall quality may be caused by the cacao seeds within the fermentation mass not being harmonized with the size of the beans or pulp quantity (LIEBEREI et al., 2010).

Different methods and vessels can be used for fermentation, e.g., cacao heaps on banana leaves, plastic boxes, bags, baskets, steel drums or wooden boxes (GUEHI et al., 2010; DE MELO PEREIRA et al., 2013; THOMPSON et al., 2012). The material as well as the design of the fermentation vessel plays a pivotal role in the course of fermentation (GUEHI et al., 2010; SCHWAN et al., 2014). SCHWAN et al. (2014) suggest that stainless steel or plastic would be superior to wooden boxes or baskets for reasons of cleaning, aeration and temperature control. However, according to GUEHI et al. (2010), fermentation in wooden boxes results in higher fermentation grades than fermenting in plastic boxes or in heaps. As cacao fermentation involves a complex microbial succession, the fermentation vessel material needs to be appropriate or starter cultures need to be used (DE MELO PEREIRA et al., 2013; SCHWAN et al. 2014). For wine, it is well known that aging in oak wood barrels, used as enological practice, improves the overall quality by stabilizing the color, adding organoleptic complexity through wood-inherent compounds, e.g., polysaccharides and tannins, and increase the phenol content (DE CONINCK, et al., 2006; KYRALEOU et al., 2015). Both in wine and in cacao, phenolic compounds contribute directly or indirectly to bitterness, astringency, and color (DE CONINCK, et al., 2006). Depending on the wood type and the contact time, sensory and color parameters in wine vary distinctly (KYRALEOU et al., 2015). In contrast to wine manufacture, changes of the fermentation process though vessel properties or other external factors are not desired in cacao production.

In Peru, fermentations are mostly conducted in wooden boxes or, less favorably for quality reasons, in bags. While some farmers process their cacao directly on their farms, it has become more common to perform the fermentation in joint post-harvest facilities of cocoa cooperatives, which facilitates a more standardized handling process (pers. comm. ROBERTO GÓMEZ ALIAGA, UNODC). To ensure a separate handling of the clone CCN-51 and the more recently cultivated Trinitario clones, new fermentation facilities with wooden fermentation boxes were built in all four cocoa farmer cooperatives in and around Tocache. The type of wood for the construction of fermentation boxes was mainly dictated by price and availability, rather than based on the suitability of different wood types for the fermentation process (pers. comm. ROBERTO GÓMEZ ALIAGA, UNODC). The properties of

wood are characterized by porosity (fine or coarse structure), anisotropy (anatomic directions), hygroscopicity and density. Wood properties are mainly species-specific, but also vary depending on the origin of the wood (BOSSHARD, 2013; ZOBEL AND BUIJTENEN, 2012). The impact of wood characteristics on the fermentation process so far has not been considered by practitioners (pers. comm. ROBERTO GÓMEZ ALIAGA, UNODC) or in science.

This study explores the fermentation characteristics of different Trinitario clones in single clone micro-fermentations. The aim was to identify clones with extraordinary properties that would merit individual marketing. For those clones that ferment in a similar manner, appropriate fermentation procedures should be developed to ensure an optimal fermentation condition when fermented together. For each clone (ICS-1, ICS-6, ICS-39, ICS-95, TSH-565, UF-613, along with the bulk clone IMC-67), separate experimental fermentations were executed both in insulated and non-insulated wooden boxes and analyzed the fermentation process and the quality of fermented and dried raw cocoa beans. In addition, the influence of the type of wood the vessel was made of on the fermentation process and the quality of raw cocoa beans was analyzed using a second approach based on mixed clone fermentations.

In particular, the following questions were assessed: (1) Do the clones ferment differently with respect to their temperature profile and to formation of free amino acids? (2) Does insulation of the micro-fermentation boxes with polystyrene change the course of fermentation and the degree of fermentation of the raw cocoa beans? (3) Do different wood types influence the course of fermentation with respect to temperature profile and quantity of amino acids in the beans?

4.3 Materials and Methods

4.3.1 Study site

Pilot-scale fermentations were carried out from 2011 to 2012 at the post-harvesting facilities of cooperative CAT (Cooperativa Agroindustrial Tocache, in 2019 name change to “Cacao Tocache”) located approximately 3 km in north east direction from the center of Tocache, in the hamlet “Bajo Almendras” (S8°09'59.1", W76°31'43.5") (see Fig. 10: FS 1). The premises of the cooperative consisted of a clonal garden, fermentation facilities for CCN-51 together with solar pre-drying and drying facilities. As the fine and flavor clones had only just been cultivated, separate fermentation facilities for those clones were not yet available.

The micro-fermentation boxes were placed inside of empty CCN-51 fermentation boxes to take advantage of existing microbial flora and as protection against cooler temperatures and wind. The

CCN-51 fermentation facilities consisted of three vertical and four horizontal box rows that were arranged stepwise (Fig. 20a). For handling purposes, the bottom boxes were used.

For drying, two different compartments existed, one with roof and sliding tray, the other with concrete floor and without roof. Tarpaulins were placed on the concrete floor to dry the beans during the day. At night, they were arranged on wooden boards in a drying facility with fixed roof.

4.3.2 Study design

Cacao pods for fermentation were obtained from cacao farmers participating in the UNODC project "Cacao instead of coca" in the province of Tocache during the harvest periods in 2011 and 2012. For single clone fermentations, pods from defined clones were harvested, opened and put into micro-fermentation boxes separately. For mixed micro-fermentations, a mixture of the Trinitario clones ICS-1, ICS-6, ICS-60, ICS-39, ICS-95, IMC-67, TSH-565, UF-29 and UF-613 was used. Identification of clones was verified by agrarian agronomists and technicians of UNODC. Since trees in the fields had just started fruiting, equal quantities of each clone could not always be obtained. However, most clones showed rather similar behavior under separate fermentation conditions (see results) and thus allowed for joint fermentation.

The ripe cacao pods were harvested and opened within 24 hours. Untreated fresh seeds with pulp were transferred into fermentation boxes. The inner dimensions of micro-fermentation boxes were 30 cm x 30 cm x 30 cm = 27 L of wet cacao seeds (approx. 9 kg of raw cocoa beans). However, the boxes were not completely filled but covered with banana leaves to provide insulation of the mass and enable the first inoculum.

The fermentation boxes were made of four different types of wood: *Cedrelinga cateniformis* Ducke (Fabaceae; common name: Tornillo), *Micrandra spruceana* (Baill.) R.E.Schult. (Euphorbiaceae; common name: Higuerilla), *Terminalia oblonga* (R.&P.) Steud. (Combretaceae; common name: Rifari) and *Cedrela odorata* L. (Meliaceae; common name: Cedro) (TANDAZO INFANTE, 2008; SERFOR, 2016). Henceforth, it is referred to the wood types with their common names. For the single clone micro-fermentations, boxes of higuerilla, rifari and tornillo were randomly selected; for the mixed fermentations, cedro boxes were also used. The effect of wood type on the fermentation, was coincidentally discovered during the single clone fermentations, so that this parameter was systematically studied during the mixed fermentation set-up. Furthermore, some boxes were used with and others without polystyrene boxes providing insulation (Fig. 20b). The clone-wise micro-fermentations were performed with or without insulation to almost equal parts, whereas the mixed fermentations were mainly done in insulated boxes. Therefore, the possible effect of insulation exclusively in the equally distributed single clone fermentations was studied. All fermentation boxes

had ten holes each of 1 cm in diameter at the bottom part of the box. The boxes were placed on wooden strips to enable free drainage of fruit pulp. During the first three days banana leaves lined the side walls and top of the boxes for inoculation and insulation purposes. Cocoa masses were mixed twice during the course of fermentation within the same box. First mixing was undertaken when alcohol odor was perceivable, second mixing when temperature started decreasing after the first temperature peak.

The end of each fermentation was based on the formation of color of the seeds and on the decreasing temperature of the fermentation mass. Samples of each fermentation box were dried in separated sections of a tray for further analyzes. Remaining raw cocoa beans were sun-dried on white tarpaulins until reaching a residual water content of 6–8%.

Figure 20: **a** CCN-51 fermentation facility of the cooperative CAT (Cooperativa Agroindustrial Tocache) in Tocache, San Martín, Peru; **b** pilot-scale cacao fermentations in micro-boxes with polystyrene insulation inside the commercial CCN-51 boxes during temperature measurement.

4.3.3 On-site analysis

The temperature was measured twice a day in the middle of each fermentation box with a fermentation thermometer (Bitherma, Kl. 1, Lemgo, Germany, total length 60 cm). The pH value of the pulp was determined once a day from a single sample per box using a pH-meter (HI98103 Checker, Hannah Instruments, Vöhringen, Germany): three cacao seeds were taken from approximately 10 cm below the surface of the mass and mixed for 15 seconds in 20 mL of purified water in a 50 mL Falcon tube. The fermentation quality was analyzed by the cut test: At the beginning of fermentation 10 beans per box were selected daily and cut and dried. At the end of the fermentation, this increased to 30 beans. Beans were cut longitudinally with a knife and examined for quality (DEL BOCA, 1962; GUEHI et al., 2010).

4.3.4 Laboratory analyses

4.3.4.1 Chemicals and standards

Unless otherwise specified, all chemicals used were obtained from Merck (Darmstadt, Germany) and were of analytical grade. HPLC standards for the analysis of amino acids were provided by Sigma/Fluka (Sigma-Aldrich Chemie/Fluka, Taufkirchen, Germany). For deionizing of water, an Elga water purification system (PURELAB Option, Elga, UK) was used.

4.3.4.2 Degreasing of cacao seeds

For each experimental trial, aliquots of the fermented and dried seed samples were placed in plastic bags and transported to the lab in Hamburg. Samples were freeze-dried for 72 h (SciQuip Ltd., Christ Alpha I-6, Newtown, UK) before manually removing seed shells of the raw cocoa beans. About 2 g of each cotyledon sample was degreased by adding 10 mL of *n*-hexane and micronized in a ball mill (MM200, Retsch, Haan, Germany) for 10 min, at a frequency of 20 s^{-1} to yield a particle size of approx. 1 μm^3. The grist was washed three times with 25 mL of petroleum ether (boiling range 40–60 °C) using a 0.45 µm filter in a Büchner funnel. The fat-free filter cake was dried at ambient temperature for 1 h and 100 mbar in a vacuum oven (Hereaus, Hanau, Germany).

4.3.4.3 Extraction and chromatographic analysis of amino acids

Extraction and analysis of free amino acids were performed according to the method described by ROHSIUS et al. (2006) with slight modifications: 0.3 g polyvinyl-polypyrrolidon (PVPP) and 10 mL of deionized H_2O were mixed with 0.1 g defatted cocoa powder to remove polyphenols. With 50% aqueous trifluoroacetic acid solution the pH was adjusted to 2.5 by stirring the solution at <4 °C for 1 h. The samples were centrifuged for 10 min at 4100 rpm (Thermo Scientific, Mega-Fuge 11 R Centrifuge, Heraeus, Hanau, Germany) and the clear supernatant filtered using a 0.45 µm syringe filter 30 µL of each sample was lyophilized (1 h; −20 °C, 0.05 mbar) and stored at −20 °C before analysis. RP-HPLC was used for subsequent sample preparation and quantitative determination of free amino acid according to ROHSIUS et al. (2006). Measurement of samples was included calibration series of the respective amino acids at λ_{ex} = 334 nm and λ_{em} = 425 nm. Amino acids were measured and quantified individually and summed up afterwards.

4.3.5 Statistics

All statistical tests were carried out with R, version 3.1.1 (R DEVELOPMENT CORE TEAM, 2014).

Differences between clones, wood types, and insulation versus temperature regime, fermentation grades and quantities of amino acids were assessed using analysis of variance (ANOVA) (QUINN AND KEOUGH, 2003). Since the fermentations were carried with a limited number of boxes for several consecutive weeks with varying weather conditions, the temporal effect was tested prior to

modeling. In the event that the experimental week had a significant effect on the respective response variable, we included the week as a factor into the models (for single clone fermentations: model on fermentation degree; for mixed fermentations: models on temperature after 48 h, temperature rise after 48 h, fermentation degree, and content of free amino acids after 144 h). Significant differences in clone or wood type were further studied by performing a Tukey HSD post-hoc test.

4.4 Results

This study showed significant differences in parameters that determine the course of fermentation for some of the clones investigated. Furthermore, the insulation of the fermentation boxes influenced temperature changes during the fermentation process. For mixed fermentations, this study showed for the first time the significant influence that different types of wood as vessel material has on the fermentation process.

4.4.1 Single clone pilot-scale fermentations

Clones showed a significantly different temperature rise during the first 48 h, when the type of wood was included in the model (clone: Df = 8, F = 2.42, p = 0.0321; wood type: Df = 2, F = 4.95, p = 0.0123). However, only ICS-95 differed significantly from UF-613 and from IMC-67 (Fig. 21a). ICS-95 showed a steeper rise in temperature than the other clones. In addition, ICS-95 showed marginal differences to ICS-60, UF-29 and TSH-565 according to the post-hoc test. Other clones did not reveal any significant differences in fermentation parameters. Differences in types of wood in respect to the temperature increase during the first 48 h were related to boxes made of rifari. These showed a significantly faster temperature increase than tornillo (Fig. 21b). Furthermore, clones differed significantly in the degree of fermentation as measured by the cut test at the end of fermentation (Df = 5, F = 3.43, p = 0.0251), with TSH-565 displaying a markedly higher value of fermentation degree than IMC-67 (Fig. 22).

The type of wood also proved to influence additional fermentation parameters, such as the temperature at 96 hours (Df = 2, F = 4.99, p = 0.0109) and the rise in temperature between 48 and 96 hours, when polystyrene insulation was included in the model (wood type: Df = 2, F = 4.12, p = 0.0227; insulation: Df = 1, F = 10.01, p = 0.0027), with rifari being substantially higher than tornillo. The temperature change between 96 and 144 hours was also significant and related to wood type together with insulation (wood type: Df = 2, F = 11.99, p < 0.0001; insulation: Df = 1, F = 11.65, p = 0.00146). However, rifari showed a decrease and tornillo an increase in temperature. In respect to

the total temperature increase between 0 and 144 hours, the influence of the wood type was indicated by a higher rise for higuerilla than for tornillo in the post-hoc test (Df = 2, F = 4.46, p = 0.0174).

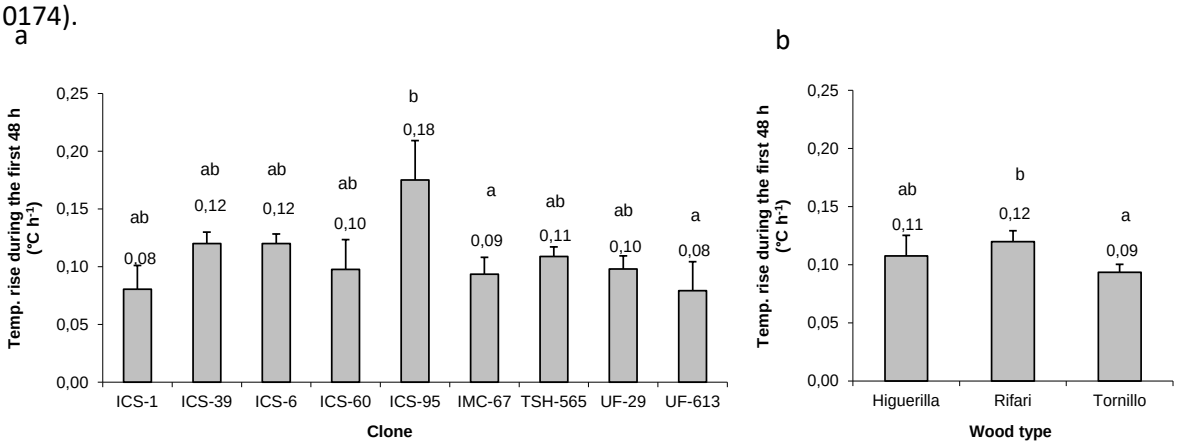

Figure 21: Temperature increase in the fermentation mass for single clone micro-fermentations during the first 48 hours in respect to **a** cacao clone and **b** wood type of fermentation boxes. (n = 49; lower case letters refer to significant differences in ANOVA followed by Tukey HSD post-hoc test; stated values display mean values; error bars show standard error).

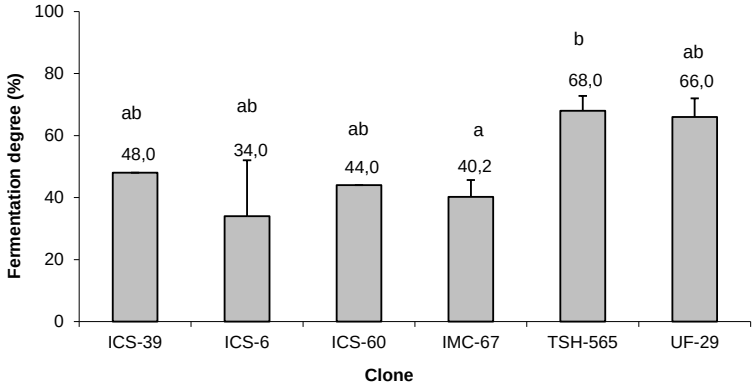

Figure 22: Degree of fermentation determined by the cut test of raw cocoa beans of different cacao clones obtained during single clone micro-fermentations. (n = 49; lower case letters refer to significant differences in ANOVA followed by Tukey HSD post-hoc test; stated values display mean values; error bars show standard error).

The mean temperature profile varied significantly when the wooden boxes were insulated with additional polystyrene foam (Fig. 23). With insulation, temperatures were more elevated during the first 72 hours, less elevated between 72 and 108 hours, and followed by equal temperature patterns between 108 and 132 hours. Thereafter temperature in boxes without insulation clearly dropped, whereas boxes with insulation maintained the temperature with another increase after 168 hours. Fermentations without insulation were terminated after 156 hours to avoid development of molds. In accordance with these temperature patterns, the statistical models revealed significantly lower temperature rise between 48 and 96 h and a significantly higher rise between 96 and 144 h with insulation (see model documentations above). In summary, during the single clone micro-

fermentation experiments the maximum average fermentation temperature was 44.2 °C (with a minimum of 38 °C; and maximum of 49 °C) with only three out of 49 boxes reaching less than 40 °C.

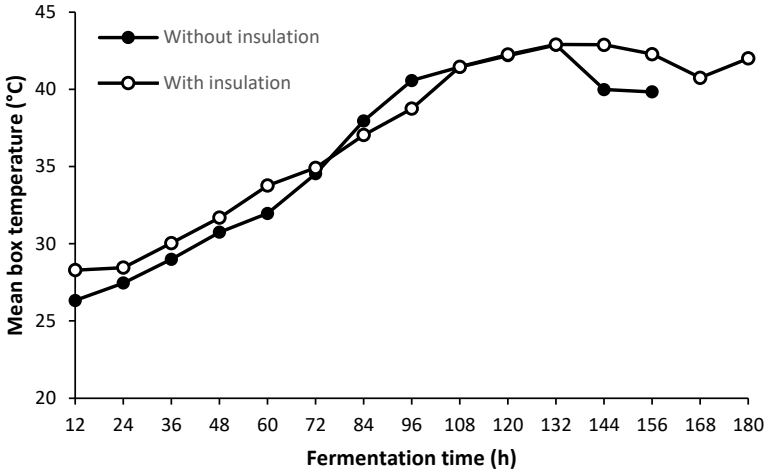

Figure 23: Mean box temperature versus fermentation time of single clone micro-fermentations with and without polystyrene insulation (n = 49).

4.4.2 Mixed pilot-scale fermentations

During the first 48 hours, in mixed micro-fermentations, using a model that included the week of fermentation, the type of wood demonstrated a clear effect on to the mean temperature increase, (wood type: Df = 3, F = 7.55, p = 0.0005; week: Df = 5, F = 8.47, p < 0.0001). Cedro was significantly different from higuerilla and rifari, and higuerilla different from tornillo. Cedro showed the steepest temperature increase followed by tornillo whereas higuerilla displayed the smallest increase (Fig. 24).

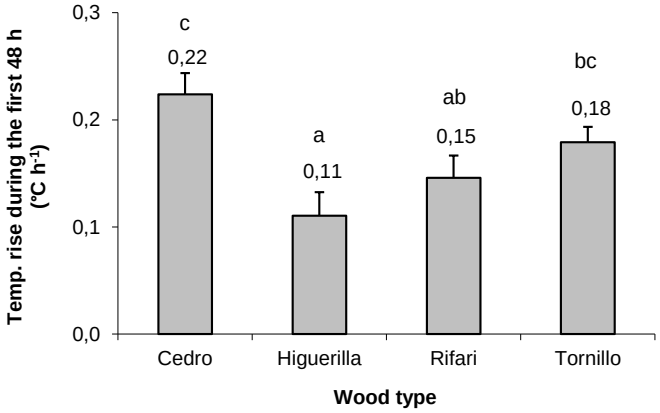

Figure 24: Temperature increase versus wood type of micro-fermentation boxes during the first 48 hours of mixed micro-fermentations (Cedro: *Cedrela odorata* L.*;* Higuerilla: *Micrandra spruceana* (Baill.) R.E.Schult.; Rifari: *Terminalia oblonga* (R.&P.) Steud.; Tornillo: *Cedrelinga cateniformis* Ducke) (n = 41; lower case letters refer to significant differences in ANOVA followed by Tukey HSD post-hoc test; stated values display mean values; error bars show standard error).

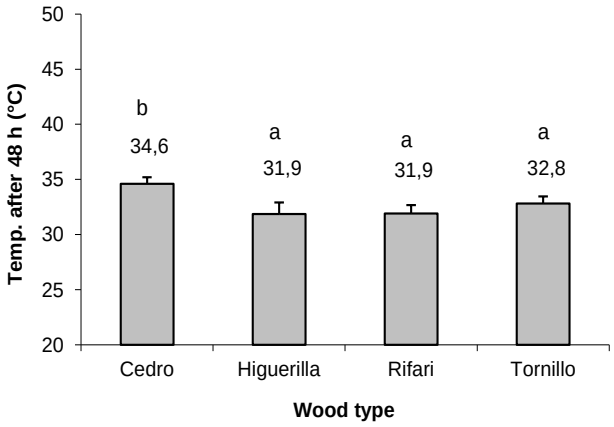

Figure 25: Mean temperatures after 48 hours of mixed micro-fermentations versus wood type of micro-fermentation boxes (Cedro: *Cedrela odorata* L.*;* Higuerilla: *Micrandra spruceana* (Baill.) R.E.Schult.; Rifari: *Terminalia oblonga* (R.&P.) Steud.; Tornillo: *Cedrelinga cateniformis* Ducke) (n = 41; lower case letters refer to significant differences in ANOVA followed by Tukey HSD post-hoc test; stated values display mean values; error bars show standard error).

After 48 hours of fermentation, mean temperatures varied significantly with the type of wood of the box. The mean temperature of cedro (34.6 °C) was higher than all other type of woods (wood type: Df = 3, F = 4.46, p = 0.0100; week: Df = 5, F = 11.11, p < 0.0001). The remaining wood types showed no significant differences between each other (Fig. 25). In contrast, the temperature increases between 48 and 96 hours (with higuerilla behaving differently from cedro and rifari) and between 96 and 144 hours (with rifari and tornillo behaving differently) were marginally influenced by the wood type. Furthermore, the maximum fermentation temperature in the boxes was related to the wood type (Df = 3, F = 3.91, p = 0.0161). In this case, higuerilla produced significantly higher values (47.5 °C) than all other types of woods (Fig. 26). The average maximum box fermentation temperature was 43.4 °C (with a of minimum 38 °C; and maximum of 49.5 °C) with seven out of 41 boxes showing values below 40 °C.

In summary, the mean temperature courses proved to differ notably among types of wood (Fig. 27). Cacao mass in boxes made of cedro increased rapidly in temperature at the beginning of fermentations but remained behind during the aerobic acidification phase. In contrast, cacao mass in higuerilla boxes was characterized by smooth temperature increase during the first fermentation hours. Subsequently, during the aerobic phase, temperatures were more elevated than in all other box types reaching the highest maximum fermentation temperatures. In comparison, cacao mass in both rifari and tornillo boxes had the lowest overall temperature regime although cacao mass in tornillo and rifari boxes showed a higher temperature increase at the beginning of fermentation than cacao mass in higuerilla boxes.

In addition, the degree of fermentation determined by the cut test was significantly influenced by the type of wood (wood type: Df = 3, F = 5.98, p = 0.0026; week: Df = 5, F = 2.24, p = 0.0763), with higuerilla showing a significantly higher mean fermentation degree in the post-hoc test than cedro and tornillo (Fig. 28).

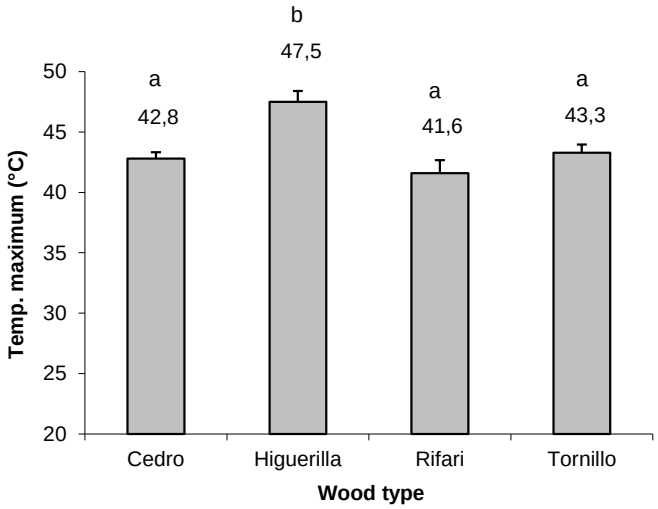

Figure 26: Mean maximum box temperatures of mixed micro-fermentations versus wood type (Cedro: *Cedrela odorata* L.*;* Higuerilla: *Micrandra spruceana* (Baill.) R.E.Schult.; Rifari: *Terminalia oblonga* (R.&P.) Steud.; Tornillo: *Cedrelinga cateniformis* Ducke) (n = 41; lower case letters refer to significant differences in ANOVA followed by Tukey HSD post-hoc test; stated values display mean values; error bars show standard error).

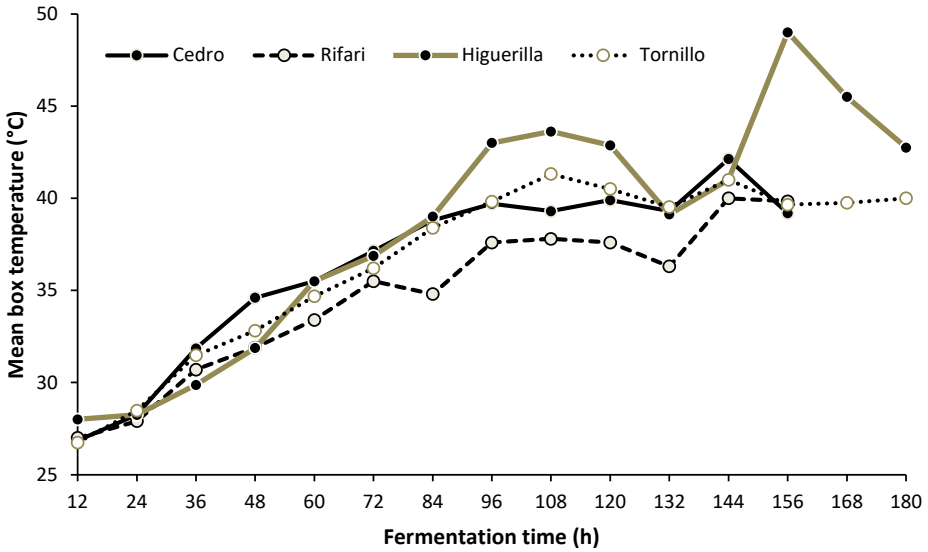

Figure 27: Mean box temperature regime and fermentation time of mixed pilot-scale fermentations with different wood types (Cedro: *Cedrela odorata* L.*;* Higuerilla: *Cedrelinga cateniformis* Ducke; Rifari: *Terminalia oblonga* (R.&P.) Steud.; Tornillo: *Cedrelinga cateniformis* Ducke) (n = 41).

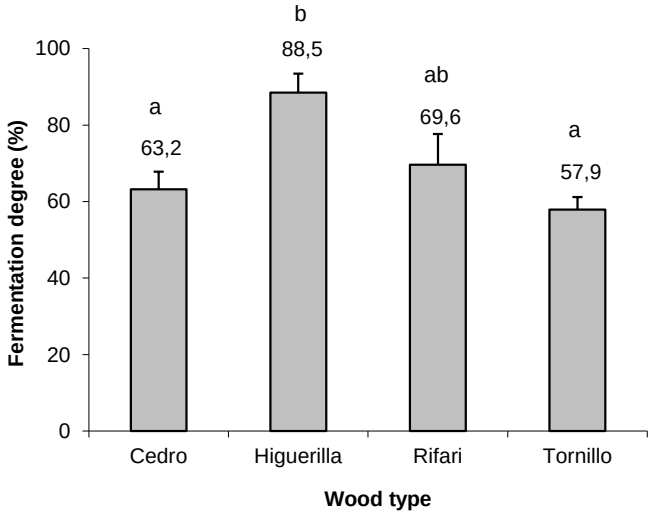

Figure 28: Fermentation degree of raw cocoa beans of mixed micro-fermentations versus wood type (Cedro: *Cedrela odorata* L.*;* Higuerilla: *Micrandra spruceana* (Baill.) R.E.Schult.; Rifari: *Terminalia oblonga* (R.&P.) Steud.; Tornillo: *Cedrelinga cateniformis* Ducke) (n = 41; lower case letters refer to significant differences in ANOVA followed by Tukey HSD post-hoc test; stated values display mean values; error bars show standard error).

The total amino acid content of raw cocoa beans was analyzed by taking samples from a subset of fermentation boxes made from cedro and tornillo on the sixth day of fermentation. The values obtained varied significantly depending on the wood type of the box (Df = 1, F = 14.09, p = 0.0024). Cocoa beans fermented in cedro boxes displayed a much higher concentration of total amino acids than those fermented in tornillo boxes (Fig. 29).

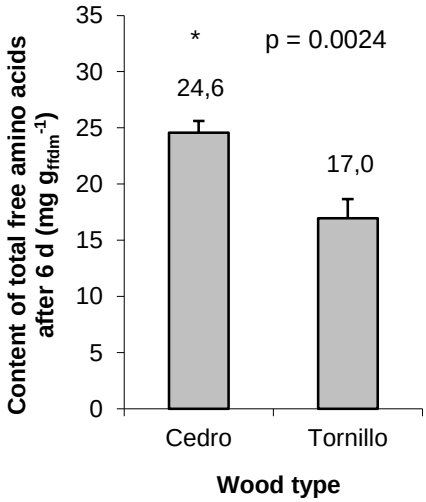

Figure 29: The content of total free amino acids after 6 days of fermentation versus wood type of boxes in mixed micro-fermentations (Cedro: *Cedrela odorata* L.*;* Tornillo: *Cedrelinga cateniformis* Ducke) (n = 16; stated values display mean values; error bars show standard error).

4.5 Discussion

In this study, the fermentation behavior of single fine and flavor clones was studied by using pilot-scale micro-fermentations. In addition to clone-specific patterns, box insulation turned out to change the temperature course of fermentation. Surprisingly, also a significant influence of the wood types, the fermentation boxes were made of, on the fermentation process on both single clone and mixed fermentations was found—an aspect that to the best of knowledge has never been studied before. The results of this study are important for the Peruvian smallholder cacao farmers that only recently started cultivating Trinitario fine and flavor cocoa. As until now there is a lack of experience on adequate post-harvest treatments in Peru, this study may represent a step forward in developing adapted fermentation techniques for achieving raw cocoa bean quality that yields high prices on the international market.

4.5.1 Micro-fermentations

For regular fermentations, it is recommended not to use less than 100 kg of wet seeds as lower amounts may not reach the desired temperatures of up to 50 °C and may therefore lead to unsatisfactory development of aroma precursors (BCCCA, 1996). However, for experimental purposes pilot-scale fermentations are common, although these should not have less than 50 kg of wet seeds and a low surface to volume ratio to minimize heat loss (BCCCA, 1996). Fermentation of smaller cacao quantities has been recommended to be conducted in net bags put into a larger fermentation mass (BCCCA, 1996). Alternatively, smaller volumes are often fermented on a laboratory scale using fermentation-like incubations under controlled temperature, acidity and/or microbial conditions (e.g., BIEHL AND PASSERN, 1982; KADOW et al., 2015; DE MELO PEREIRA et al., 2012; STOLL, 2010). However, such approaches show several disadvantages that hinder the transfer of the obtained results into practice. In particular, it can be assumed that a foreign cacao mass surrounding the studied clones, as performed with cacao seed samples in net bags within larger fermentation masses (BCCCA, 1996), strongly influences the course and flavor profile. Accordingly, KADOW et al. (2013) found that pulp-inherent volatile compounds penetrate into the seed after approximately two days of fermentation.

Consequently, it was decided to ferment only small amounts of approximately 20 kg of wet cacao seeds in wooden boxes for the following strengths of this approach: (1) The experimental set-up mimics regular fermentations at local microbiological and environmental conditions; (2) It allows for using fresh cacao seeds for these evaluations; (3) The fermentation mass of single cacao clones was protected from the influence of other genetic cacao material and microbes.

In total, 90 micro-fermentations were carried out with a mean maximum temperature of 43.8 °C, with 80 boxes showing a maximum temperature of more than 40 °C. According to BIEHL AND PASSERN (1982), increased proteolytic activity can be observed already at 40 °C, which means that aroma precursors have likely been formed (see Fig. 29). Hence, the experimental set-up using micro-boxes, partly with polystyrene insulation, seems to be suitable for experimental fermentations. Due to the fact that cacao trees in the study area only recently started fruiting, in some cases the material was not sufficient to fill the fermentation boxes entirely. This may be one reason why some boxes showed maximum temperatures below 40 °C. In some cases, however, also unfavorable weather conditions seemed to prevent vigorous fermentation (pers. observation).

4.5.2 Single clone pilot-scale fermentations

Overall, temperature development during single clone micro-fermentations indicates successful fermentation in most cases (46 of 49 boxes) with maximum fermentation temperatures over 40 °C. Temperature rise during the first 48 h was in general similar between clones, except for ICS-95 showing a significantly faster increase (Fig. 21a). This may be attributed to reduced fruit pulp that may have led to acceleration of fermentation (SCHWAN AND WHEALS, 2004)—the reason for pulp removal in Brazil. However, these data did not reveal significant differences in pulp/seed ratio of ICS-95 in comparison with the other clones (Chapter 3, Tab. 3), although this clone according to visual assessment seemed to have less pulp (own observation). Also seed size of ICS-95 did not vary significantly and can therefore not be an explanation (Chapter 3, Tab. 3). Interestingly, the temperature regime during the initial fermentation seemed to be influenced by wood type to the same degree as by the clones themselves (Fig. 21b). This points to rather weak clone-specific behavior, especially since the temperature rise during the first fermentation hours did not affect the final fermentation result.

Concerning the fermentation success, TSH-565 showed a significantly higher mean fermentation degree determined by cut test than the Forastero clone ICS-67 without wood types being significant in that model, and no further difference among the studied Trinitario clone types were detected (Fig. 22). The Forastero IMC-67 originates from the Upper Amazon region in Peru, whereas TSH-565 is a crossing between SCA-6 (Scavina-6: Forastero native to the Upper Amazon region in Peru) and ICS-1 (Trinitario) classified as fine and flavor cocoa (GARCÍA CARRIÓN, 2012; ICGD, 2020). In general, Forastero clones need a prolonged fermentation time for the formation of aroma precursors compared with Trinitario clones (LIEBEREI et al., 2010) and hence may have been expected to show among the lowest fermentation degree of all clones analyzed. In contrast, also the three ICS clones (Trinitario cultivars) exhibited low to intermediate fermentation degrees, and exclusively UF-29

(hybrid of Nacional × unknown) had an almost equally as high fermentation degree as TSH-565. Consequently, fermentation degree of all clones was rather low compared with commercial standards (BCCCA, 1996). The same often applies to commercial-scale fermentations in the study region, which implies that low fermentation degrees in this study are not only a consequence of box size. For this reason, quality criteria with respect to fermentation grade of fine and flavor clones are currently in general under discussion as purple beans often do not possess astringed and bitter aroma profiles in comparison to Forastero clones—presumably owing to different binding behavior of polyphenols in fine and flavor versus Forestero cacao (pers. comm. REINHARD LIEBEREI).

The single clone fermentations revealed certain evidence of the effect of vessel material on the fermentation course. Usually, fermentation of small cacao quantities is conducted in either plastic or steel containers under controlled lab conditions. However, on a commercial scale, GUEHI et al. (2010) emphasized that fermentation in wooden boxes results in higher fermentation grade than fermenting in plastic boxes or heaps. As stated above, there were so far no studies on the effect of different wood types of fermentation boxes. Even though this part of the study did not rely on a balanced design which tested the different wood types systematically, these results indicate a possible influence of the wood type on the course of fermentation. In particular, mean temperature rise during the first 48 h and between 48 and 96 h was significantly higher in boxes made of rifari than in those of tornillo. In contrast, between 96 and 144 hours tornillo showed a temperature increase and rifari a decrease. Overall mean temperature increase from 0 to 144 hours, however, was most pronounced in boxes made of higuerilla. Despite these findings, the results seem rather be connected with insulation using tapped polystyrene boxes (Fig. 23). In the set-up, all boxes made of higuerilla were insulated, whereas insulation was missing in the rifari boxes and most of the tornillo boxes. Interestingly, the steepest initial temperature was measured in the non-insulated rifari boxes, which points to a possibly negative effect of polystyrene boxes, which due to the tap hampered both natural inoculation with microorganisms which are essential for the course of fermentation (e.g., BCCCA, 1996; SCHWAN AND WHEALS, 2004) and pronounced aeration of the mass during the acidification phase. Once boxes reached higher temperatures, this was maintained by the insulation whereas in boxes without insulation temperature dropped. This may explain the overall highest temperature of the insulated higuerilla boxes. Based on the findings of possible effects of wood type on fermentation, the mixed micro-fermentations of the second part were analyzed.

4.5.3 Mixed pilot-scale fermentations

The mixed micro-fermentations, that systematically tested different types of box material, consolidated the assumption that timber significantly influenced the temperature regime during

fermentation. As cedro was additionally included in the analyses, models show different relations, albeit key facts remain similar. The influence of the timber types may be attributed to specific wood properties of the different species. In boxes made of cedro, temperature rise was significantly steeper at the beginning of fermentation, whereas in the further course of fermentation, temperatures in those boxes did not reach the high maximum temperatures found in boxes made of higuerilla (Fig. 24, 25, 26, 27). All trees used for fermentation boxes are native to tropical America (SERFOR, 2016), which makes finding comparable data on wood traits complex. As in all tree species, raw density (related to cell wall substance (mass) and the proportion of cavities in a given wood volume) and other properties vary according to environmental conditions and treatments (GESAMTVERBAND DEUTSCHER HOLZHANDEL E.V., 2020). Cedro, known as Spanish-cedar, is the most commercially used species in the genus *Cedrela* (CINTRON, 1990). Cedro is under threat due to ongoing exploitation and therefore listed as "vulnerable" on the red list of endangered species (IUCN, 2020). Furthermore, trade is restricted in Colombia, Guatemala, and Peru by the "Convention on International Trade in Endangered Species of Wild Fauna and Flora" (CITES, 2020). The species is frequently planted in agroforestry systems together with coffee and cacao (LEMMENS, 2008). Its wood is of light to medium weight with a raw density ranging between 260–525 kg $(m^3)^{-1}$ at 12% moisture content. The timber has a slight risk of cracking and deformation. It is used for cigar boxes, music instruments, plywood, etc. (LEMMENS, 2008). The wood of cedro has an insect-repellent smell. In addition, it is used to extract a volatile oil. In view of these findings, it can be suggested that cedro has high insulation properties due to intermediate density and a high content in volatile oils. No evidence was found that the timber-inherent substances influence cocoa bean flavors, as the production of free amino acids was even higher than in tornillo. However, as the resulting flavor was not analyzed in this study, further research on this issue seems necessary.

The evergreen tree higuerilla is characterized by large buttress roots and contains low amounts of a white latex that is low in rubber and high in resin. It is used for decorative paneling (TROPICAL PLANTS DATABASE, 2020a), even though scarcely, but very little is known about this tree. Its fruits are used as food by the indigenous population of the Amazon basin (SOUZA, 2012). According to these observations, higuerilla timber had good properties to support the fermentation process, in particular concerning high maximum temperatures and fermentation (Fig. 26, 27). The boxes showed a more pronounced heat transfer, indicated by a higher temperature at the exterior during fermentation (pers. observation), but it remains an open question which properties caused the changes in fermentation. Moreover, timber did not tolerate extreme temperature and moisture amplitudes which led to strong deformation and cracks of the boxes. Therefore, it seems less appropriate for long-term usage as fermentation box material when material traits cannot be improved using seasoned rather than fresh timber. Overall, further studies are needed to confirm

these findings also for large commercial-scale boxes with a different temperature and moisture regime due to smaller surface to volume ratio.

Rifari fermentation boxes displayed intermediate properties with regard to temperature rise, maximum fermentation temperatures and resulting fermentation degree of cocoa mass (Fig. 25, 26, 28). Heartwood of rifari is yellow with a medium texture and a high to very high density. A distinctive aroma or smell is not noticeable. The tree is harvested directly in the rainforest or produced as a fast-growing plantation tree. Its timber is used for furniture, floors, ship building, etc. (MOYA AND MUÑOZ, 2010; TROPICAL PLANTS DATABASE, 2020b). In addition to the displayed results, a pronounced condensation inside the boxes made of rifari timber was observed which led to severe molds in the fermentation mass. Rifari has a very lease, closed surface that hampers aeration (own observation). Therefore, rifari timber cannot be recommended for producing cacao fermentation boxes. Again, further investigations on regular fermentation scale may elucidate rifari timber box properties and its suitability for cacao fermentation.

With regard to the fermentations executed in boxes made of tornillo timber, this study showed intermediate temperature rise during the first 48 h, intermediate temperature after 48 hours as well as an intermediate mean temperature maximum (Fig. 24, 25, 26). However, the fermentation degree of raw cocoa beans was lowest and also the formation of total amino acids after six days was significantly less pronounced compared with cacao fermented in boxes of cedro wood (Fig. 27, 28). Tornillo is commonly harvested from the rainforest or planted in plantations (TROPICAL PLANTS DATABASE, 2020c). Its heartwood is light brown, texture is coarse, and odor or taste are absent in dried wood. Timber is moderately hard to very hard, but at the same time elastic. The density is intermediate with a density of 640 kg $(m^3)^{-1}$ of air-dried material collected in Brazil and about 480 kg $(m^3)^{-1}$ of timber from Peru (TROPICAL PLANTS DATABASE, 2020c). Tornillo timber is widely used for construction, furniture, etc. (FERNANDES et al., 2018; TROPICAL PLANTS DATABASE, 2020c) and is not protected under CITES regulations (CITES, 2020). In this study, fermentation boxes made from tornillo timber were resistant to heat and sweat water; also, fermentation mass did not show molds. In particular in Peru, tornillo timber is often used for the construction of fermentation boxes (pers. comm. ROBERTO GÓMEZ ALIAGA, UNODC). However, data show that cocoa beans fermented in other types of timber resulted in better results in terms of fermentation degree and formation of free amino acids, which again underpins the need for further investigations with regular scale fermentations.

4.6 Conclusion

This study provides evidence that fermentation in timber micro-boxes is possible and leads to adequate and comparable results. Analyses revealed that few clone-specific patterns exist, whereas insulation and fermentation box timber types clearly alter the course of fermentation and hence the quality of raw cocoa beans. The comparison of clones showed minor differences of ICS-95 from the other clones in fermentation temperature increase during the first 48 h, which did not translate to final fermentation degree obtained by cut test as here, TSH-565 was significantly better fermented than ICS-67. Insulation with polystyrene boxes significantly changed the fermentation process, but surprisingly did not lead to better results as the temperature rose more slowly, most probably owing to reduced inoculation and aeration of the fermentation mass. Exclusively from 132 hours of fermentation onwards, insulation prevented a quick heat loss, which is not expected to be related to cocoa bean quality parameters. Despite the partly unbalanced study design, both pilot-scale set-ups were suitable to detect significant differences regarding the analyzed timber types. Box timber type proved to influence the temperature regime of the fermentation with higuerilla (*Micrandra spruceana* (Baill.) R.E.Schult) having the most pronounced temperature increase over the whole course of fermentation (0 – 144 h) and the highest mean maximum temperature. Moreover, the fermentation degree of raw cocoa beans showed the highest values when fermented in higuerilla boxes. However, taking merely the statistical data into consideration for timber type decision, would be misleading as own observations revealed deformation and breaking of higuerilla boxes due to temperature and moisture amplitude. Interestingly, cacao mass fermented in boxes of Cedro (*Cedrela odorata* L.) showed a steep temperature rise and also maintained elevated temperatures at the end of fermentation, albeit maximum temperature was significantly lower than in higuerilla. Rifari timber (*Terminalia oblonga* (R.&P.) Steud.) had intermediate properties, but tended to develop heavy mold infestations during the course of fermentation and is therefore not recommendable. Tornillo timber showed intermediate to rather poor influence on the fermentation process with regard to temperature regime, the fermentation degree and the formation of free amino acids in cocoa beans. However, Tornillo timber is commonly used in Peru for the construction of cacao fermentation boxes. On farm and cooperative level, construction of timber fermentation vessels should be oriented in solutions that are cost efficient and easy to handle. Therefore, these results suggest to further investigate on adequate timber for fermentation box construction to enhance cocoa bean quality and thus farmer income by optimizing the fermentation process. The study furthermore indicates that Peruvian cacao farmers may ferment newly introduced Trinitario clones, including the Forastero IMC-67, within the same boxes without compromising fermentation success due to inhomogeneous properties—owing to the rather minor differences among clones in

fermentation traits. Consequently, this study may contribute to assist Peruvian smallholder farmers based on novel insights concerning scarcely studied drivers of the fermentation process.

5 SYNTHESIS

Many studies attribute cacao yield and quality predominantly to the genetics of cacao cultivars (e.g. LIEBEREI et al., 2010; NIEMENAK et al., 2006), whereas other drivers have largely not been taken into consideration. In contrast to this view, the studies of this dissertation thesis clearly showed that environmental conditions, farm management, and adaptations of the fermentation process may be equally as important as the differences among clones typically cultivated in Peru. In particular, this work provides insights on the impact of soil, biodiversity, and farm management on cacao yield and quality. Furthermore, pulp pre-conditioning and the selection of timber for fermentation boxes proved to influence the fermentation processes and the final quality of raw cocoa beans. This information may serve as a guidance for Peruvian smallholder farmers and cacao cooperatives. Moreover, the idea of finding locally adapted solutions for cacao growing and fermentation which contribute to a higher quality seems promising for all major cacao producing regions of the world.

5.1 Interactions of environment, farm management, and cacao

Cacao is planted in diverging cultivation systems ranging from full-sun monocultures to complex agroforestry and intercropping systems. This may be attributed to the fact that the interplay between cacao trees, environmental factors, farm management actions and accompanying vegetation are as yet poorly understood. In contrast to large farms that rely on full-sun systems for yield reasons, agroforestry or intercropping systems have been considered more suitable for smallholder farmers as they contribute to diversify income and hence to secure their livelihood. This is the reason why agroforestry systems have frequently been studied since decades (e.g., WARTENBERG et al., 2020). However, agroforestry systems are believed to have negative effects on yield due to the assumed competition among crops and other plant species (see CLOUGH et al., 2011 for cacao). On the other hand, species diversity in agroforestry or intercropping systems may lead to complementary interactions among plants (SCHWENDENMANN et al., 2010; SMITH et al., 2010), which positively influence cacao yield and quality (DEHEUVELS et al., 2012) along with the robustness against pests (SABATIER et al., 2013).

The data confirmed that the cover of the shade tree layer may have a negative effect on fruit set and size of healthy, ripe pods, albeit a direct effect on cacao yield per hectare was not found. However, possible negative effects of shade tree layer on yield parameters may be eliminated by species selection and adequate tree spacing (as reported by GIDOIN et al., 2014; KOKO et al., 2013).

Moreover, harvesting shade trees may provide the farmers with additional income, which may compensate for yield losses. Depending on the species planted, cacao trees can even benefit from shade trees, e.g. by nitrogen providing legumes (HERION et al., submitted). Thus, economic long-term effects have to be included when planning a farm set-up, as it was done in the studied project of UNODC (GÓMEZ ALIAGA et al., 2014). Interestingly, also an impact of shade trees on cocoa bean quality was found (e.g., increased levels of polyphenols like epicatechin), which may enable the farmers to modify the contents of quality-relevant plant secondary compounds based on farm management. This is particularly important as chocolate consumers have increasing interest in healthy products with high contents of polyphenols.

In comparison to shade tree layer, herb and shrub layer showed clear positive impacts on fruit number per tree (as similarly reported by HERION et al., submitted and ZUG et al., 2019) and on resistance against *Phytophthora* spp. Additionally, a diverse herb and shrub layer proved to increase methylxanthine content in cacao seeds. Herbs and shrubs in cacao plantations are mainly weeds or, in the case of shrubs, also planted on purpose. The positive effects of the understory layer on yield and quality parameters underpin the widely acknowledged importance of biodiversity for human well-being (e.g., MILLENIUM ECOSYSTEM ASSESSMENT, 2005). Based on the data, the combination of productive cacao plantations with high biological diversity seems possible, thereby providing farmers with a more stable income, while improving biodiversity conservation. Again, the study proved that apart from yield also cocoa bean quality (e.g., caffeine content) responded to biodiversity, which may hence be influenced by adapting management of the herb and shrub layer. As, according to these analyses, the caffeine content also increases the resistance against pests (such as *Phytophthora* spp.), pest management and the aim of quality improvement of cocoa bean quality are closely interrelated. Consequently, this study provides a huge array of potential measures on farm level that can be included in future plantation set-ups.

5.2 Influence of pulp-conditioning prior to fermentation on the quality of raw cocoa beans

As Trinitario clones were only recently cultivated in Peru, farmers and cooperatives still require guidance in terms of adapted post-harvesting processes. In recent times, mainly the highly productive clone CCN-51 was cultivated within the department of San Martín that is characterized by high pulp amounts, which made it necessary to leave seeds in plastic boxes for pulp drainage for approx. 24 hours prior to fermentation in boxes (own observation). The studied Trinitario clones were not expected to profit from the same treatment owing to their different properties. Therefore, an array of easily applicable techniques was explored in this study. Furthermore, pulp quantity and

quality have been reported to rely on farming and environmental conditions (HEGMANN, 2015), which highlights that fermentation process should be adapted to local conditions. The findings on pulp conditioning prior to fermentation allow farmers and cooperatives for adapting the raw cocoa quality according to customers and consumers preferences. In case that lower contents of polyphenols and amino acids are desired, pulp removal seems favorable. In contrast, pod storage is recommendable when higher contents of these substances along with the corresponding flavor profile is demanded. Overall, pulp pre-conditioning techniques proved to be valuable to modify the quality of cocoa beans and hence the market price obtained by the farmers. This is particularly important to farmers in Peru and elsewhere as the methods used for pre-conditioning were easy and did not lead to higher production costs. However, in-field methods to produce inocula that showed an effect on fermentation could not be developed. Therefore, further research is needed to efficiently manipulate the microbial environment by using locally available and cost-effective cultures to better control the fermentation process.

5.3 Influence of clone traits and fermentation vessel properties on the course of fermentation

Cacao clone traits in terms of aroma profile, pulp quantity, seed size, etc. have been reported to vary within a considerably wide range (e.g., NIEMENAK et al., 2006; KONGOR et al., 2016). However, this study showed that the Trinitario clones selected by UNODC in Tocache have very similar fermentation properties. Therefore, a separate clone-wise fermentation may not be recommended to farmers and cacao cooperatives of the study region.

In contrast, significant influences of timber types used for the fermentation boxes on the course of fermentation were found. Overall, some wood types proved to support different aspects of the fermentation process, with cedro leading to higher temperature rise at the beginning of the fermentation and higuerilla being accompanied by the highest maximum temperatures and fermentation degree. However, none of the wood types were optimal for construction of fermentation boxes. Higuerilla yielded the best fermentation results, but the timber is not adequate for fermentation as is deforms and cracks due to temperature rise and moisture even in small boxes. Cedro showed some effects at the beginning of fermentation. Nevertheless, it is listed as endangered species and should therefore not be used in large scale. Finally, the other timber types resulted in poor fermentation success. Therefore, further investigation is recommended. Again, it is crucial to search for local solutions in different cacao producing regions, which take the availability and suitability of different tree species into account.

In contrast to the prior expectations that avoiding heat losses during fermentation is mainly positive for the final product, it was found that insulation of fermentation boxes had pronounced negative effects on temperature development of fermentation. This can presumably be attributed to insufficient inoculation and aeration within closed boxes. Insulation is thus not recommended. The use of wooden boxes without amendment of further materials seems to be optimal to obtain more or less constant results of fermentation. Apart from providing inoculation (GUEHI et al. 2010), it is easily available to farmers and cooperatives worldwide and does not cause disposal problems.

5.4 Cacao production in the province of Tocache: Between ideals and realism

The studies were designed to meet farmers' and cacao cooperatives' needs concerning applicable and cost-effective solutions for cacao fermentations. It was therefore refrained from using professional starter cultures or other fermentation vessel material than timber.

All cacao farmers that were part of the UNODC project in the province of Tocache, Peru, formerly grew coca as main income source and consequently had little experience with cultivation and post-harvest treatments of the new crop and its different varieties. Other former coca farmers cultivate oil palm, coffee or peach palm as alternative crops. During the UN project, the participating farmers were trained and received technical assistance by engineers and technicians of UNODC along with fertilizer and pesticides for free. By the end of the project, farmers had to apply their new knowledge self-reliantly for maintenance of their livelihood. Most cacao farmers in the study region are organized in cacao cooperatives that support farmers with knowledge about cacao cultivation, with joint processing of cacao in collective post-harvest facilities, and with finding customers for the raw cocoa beans produced. However, the cacao farmers are not mandatory bound to the cooperatives, leading to the fact that they tend to sell their raw cocoa beans to the best paying customer (pers. comm. ROBERTO GÓMEZ ALIAGA, UNODC). This poses problems to the cooperatives concerning their economic sustainability as, in this area, each cacao cooperative has only a few hundred members (in comparison to West Africa where ten thousands of members are common). Due to the continuous shortage of financial resources, cooperatives for example tend to struggle with regard to pre-financing of cacao seeds from the cacao farmers.

Furthermore, often no price differentiation of customers is paid for bulk or fine and flavor cocoa (pers. comm. ROBERTO GÓMEZ ALIAGA, UNODC). As bulk cocoa such as CCN-51 yields higher and is more pest resistant than fine and flavor cocoa, farmers are hardly encouraged to cultivate fine and flavor clones without a surplus price paid by customers. Once many farmers would resign from cultivating fine and flavor cocoa, the classification of Peru as fine and flavor country by ICCO may be affected.

In order to establish stable incomes for the cacao farmers, one strategy is to become more independent from cocoa stock price by receiving premiums for well processed fine and flavor cocoa varieties. Another strategy comprises differentiation from the common market by certification of raw cocoa beans with e.g., organic and/or fair-trade seals—an option that is quite common in the study area. Also, growing cacao types that exist exclusively in a certain area and are characterized by unique fine and flavor traits may be a valid strategy. Accordingly, the market for single origin, "grand cru", single clone specialty chocolates is constantly growing and high prized. In Peru, already some of these cacao specialties exist, e.g., Porcelana from Piura and Chuncho from Cusco. Finally, local production of cocoa products and chocolate is being implemented by cooperatives or companies in the study region and all over Peru.

UNODC followed all stated strategies to ensure farmers' incomes in the long term (pers. comm. ROBERTO GÓMEZ ALIAGA, UNODC). Therefore, native and promising cacao trees were evaluated for their properties. Apart from yield, resistance and flavor also the tendency of cacao trees to accumulate cadmium plays a pivotal role in South America as maximum allowed values of cadmium in chocolates with a high cocoa content are often reached or exceeded (e.g., ZUG et al., 2019). After identification of promising cacao trees and their evaluation concerning the mentioned criteria, clone gardens were established to gain sufficient plant material for further examination. The field study showed several interactions between yield, soil, biodiversity, and farm management that may be applied when installing plantations with these selected promising cacao types.

Today, many agrarian universities and institutes along with several technical colleges have developed a strong focus on cacao research and formation in Peru. Therefore, cacao farmers and cooperatives may profit from a huge number of professionals who are well trained in this field and can transfer their knowledge. Cacao farmers and cooperatives need applicable and cheap solutions for the improvement and adaption of farm management and post-harvest treatments. This study aimed at meeting this need by supplying an array of different applicable solutions. Consequently, the findings of this study were communicated in several workshops to cooperatives and farmers, carried out already during the studies. However, some aspects found in this study require further investigation to untangle the complex process of cocoa production. In particular, drying subsequent to fermentation was not part of this study, even though it is known to profoundly influence on the quality traits of raw cocoa beans.

5.5 A future outlook on cacao cultivation in Peru and in the world

One of the most important challenges for mankind during the next decades is the mitigation of and the adaptation to climate change, which also holds true for cacao cultivation worldwide (e.g., LAHIVE

et al., 2019). Already during the last couple of years, profound changes in the climatic conditions in South America were notable. In the study region, particularly the course of wet and dry seasons was affected (pers. comm. ROBERTO GÓMEZ ALIAGA), which clearly endangers the growth of the highly climate-sensitive cacao tree. Therefore, cacao types resistant to both increased drought and increased water logging will be needed in future. In addition, also the classical breeding scopes of high productivity, yield efficiency, disease resistance, nutraceutical value, superior flavor and quality attributes will be in focus (BEKELE AND PHILIPS-MORA, 2019). DE SOUSA et al. (2019) highlighted the importance of agroforestry systems as key strategy towards more resilient agricultural systems. However, they also forecast for Mesoamerica that the current agroforestry systems with preferred tree species are vulnerable when climate will change. The authors therefore suggest a transformation of agroforestry systems using climate-adapted tree species compositions. In West Africa (Ghana and Ivory Coast), the impact of climate change on cacao cultivation is prospected to be particularly pronounced (LÄDERACH et al., 2013). While some areas are expected to become unsuitable for cacao cultivation others are foreseen to even increase in suitability. As possible adaptation strategy, changes in agricultural management are suggested. In accordance to the Western African situation, also the Brazilian cacao cultivation region Bahia will be subjected to increasing impacts of climate change. El Niño-Southern Oscillation (ENSO) showed in modulations to increase in frequency causing severe droughts in parts of South America. However, already in the years between 2015 and 2016 ENSO caused high tree mortality of 15%, decreased cacao yield by 89%, and increased infection rate with witches' broom (*Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora) in the state of Bahia (GATEAU-REY et al., 2018). ORTEGA ANDRADE et al. (2016) predict that the fungal disease *Moniliophthora roreri* (Cif & Par) Evans will spread to so far uninfected areas in South America supported by changed climate conditions. However, LAHIVE et al. (2019) point out that the response of different genotypes to water limitation differs considerably, which again implies to modify the classical aims of cacao breeding towards more climate-resilient varieties. In Peru, a study on survival rate of cacao trees after seasonal flood events found that younger and unshaded trees were most vulnerable to flooding (DELGADO et al., 2016). Accordingly, the authors emphasized the important role of agroforestry systems in climate change adaptation.

The findings on the complex interplay of biodiversity, pathogen incidence, and cacao yield and quality in agroforestry systems lead to a better understanding of the functioning of agroforestry systems and may therefore contribute to the necessary transition towards production systems that are more resilient to future changes.

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Figures

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Figure 2: Figure: Lieberei et al., 2010

Figure 3: Photographs: **a** + **b** J. Cierjacks

Figure 4: Photographs: **a** + **b** + **c** J. Cierjacks

Figure 5: Photographs: **a** + **b** + **c** R. Gómez Aliaga

Figure 6: Photographs: **a** + **b** + **c** J. Cierjacks

Figure 7: Photographs: **a** + **b** + **c** J. Cierjacks

Figure 8: Photographs: **a** + **b** + **c** J. Cierjacks

Figure 9: Photographs: J. Cierjacks

Figure 10: Maps: **a** Google earth, retrieved: 2016; **b** Google maps, retrieved: 2016

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
µm	Micrometer
AIC	Akaike information criterion
ANOVA	Analysis of variance
C	Carbon
CA	Correspondence analysis
CAT	Cooperativa Agroindustrial Tocache
CCN	Colleción Castro Naranjal (Cacao breeding/genebank collection)
CEC	Cation exchange capacity
cm	Centimeter
Df	Degrees of freedom
F	F-test of equality of variances
ffa	Free fatty acids
ffdm	Fat free dry matter
FS	Fermentation site
g	Gram
GLMM	Generalized Linear Mixed Models
h	Hour
ha	Hectare
ICCO	International Cocoa Organization
ICS	Imperial College Selection (Cacao genebank collection)
IMC	Iquitos Mixed Calabacillo (Cacao genebank collection)
km	Kilometer
L	Liter
lme	Linear mixed-effects model
m	Meter
mbar	Millibar
min	Minute

mL	Milliliter
N	Nitrogen
n	Number
nm	Nanometer
NMDS	Non-metric multidimensional scaling
p	“Probabilitas”-value (Significance value)
rho	Spearman's rank correlation coefficient
RP-HPLC	Reversed phase high performance liquid chromatography
rpm	Rotations per minute
s	Second
SE	Standard error
t	Tons
TSH	Trinidad Selected Hybrid (Cacao genebank collection)
Tukey HSD	Tukey’s Honest Significant Difference test
UF	United Fruit (Cacao genebank collection)
UNODC	United Nations Office on Drug and Crime
UV/VIS	Ultraviolet/visible
vs.	Versus
y	Year
λ	Lambda: Wavelength

ANNEX

Table 6: Species list of all vascular plants found on cacao plantations in the herb, shrub and shade layer as described in chapter 2 shown with their frequency of occurrence in percent of all pots. It comprises a total of 43 families; three plants families remained unknown. Identification to genus or species level was done when possible.

Plant family	Species	Frequency of occurence (% of all plots)		
		Herb layer	**Shrub layer**	**Shade trees**
Amaranthaceae	*Alternanthera sp. 1*	31.3	0.0	0.0
	Alternanthera sp. 2	2.1	0.0	0.0
	Alternanthera sp. 3	2.1	0.0	0.0
	Alternanthera sp. 4	2.1	0.0	0.0
Annonaceae	*Annona sp.*	2.1	0.0	2.1
Apocynaceae	*Tabernaemontana sananho* Ruiz & Pav.	0.0	0.0	4.2
Araceae	*Colocasia esculenta* (L.) Schott	18.8	0.0	0.0
Arecaceae	*Attalea phalerata* Mart.	0.0	0.0	8.3
	Bactris gasipaes Kunth.	0.0	0.0	6.3
	Cocos nucifera L.	2.1	0.0	4.2
	Elaeis guineensis Jacq.	6.3	0.0	16.7
	Euterpe oleracea Mart.	2.1	0.0	4.2
	Mauritia flexuosa L.f.	2.1	0.0	0.0
Asclepiadaceae	*Asclepias curassavica* L.	4.2	0.0	0.0
Asteraceae	*Ageratum conyzoides* L.	2.1	0.0	0.0
	Hymenoxys sp.	6.3	0.0	0.0
	Sonchus oleraceus L.	8.3	0.0	0.0
	Taraxacum sp.	10.4	0.0	0.0
	Vernonia sp. 1	25.0	4.2	0.0
	Vernonia sp. 2	4.2	0.0	0.0
	Vernonia sp. 3	10.4	0.0	0.0
	Asteraceae 1	2.1	0.0	0.0
	Asteraceae 2	14.6	0.0	0.0
	Asteraceae 3	2.1	0.0	0.0
	Asteraceae 4	2.1	0.0	0.0
Bignoniaceae	Bignoniaceae 1	2.1	0.0	0.0
	Bignoniaceae 2	25.0	0.0	0.0
	Bignoniaceae 3	2.1	0.0	0.0
Bixaceae	*Bixa orellana* L.	2.1	0.0	0.0
Caricaceae	*Carica papaya* L.	0.0	2.1	6.3
Cecropiaceae	*Cecropia sp.*	8.3	0.0	0.0
Commelinaceae	*Commelina sp.*	39.6	0.0	0.0
	Tripogentra sp.	6.3	0.0	0.0
Cucurbitaceae	*Cucumis sp.*	2.1	0.0	0.0
	Cyclanthera pedata (L.) Schrad.	2.1	0.0	0.0
Cupressaceae	*Cupressus lusitanica* Mill.	0.0	0.0	4.2
Cyperaceae	*Cyperus ferax* L. Rich.	6.3	0.0	0.0
	Cyperus rotundus L.	8.3	0.0	0.0
	Fimbristylis miliacea (L.) Vahl	2.1	0.0	0.0

	Fimbristylis sp.	2.1	0.0	0.0
	Scleria sp.	35.4	0.0	0.0
Erythroxylaceae	*Erythroxylum coca* Lam.	6.3	6.3	2.1
Euphorbiaceae	*Acalypha sp.*	6.3	0.0	0.0
	Euphorbia heterophylla L.	8.3	0.0	0.0
	Euphorbia hyssopifolia (L.) Small	2.1	4.2	0.0
	Ricinus communis L.	0.0	0.0	2.1
Fabaceae	*Cedrelinga cateniformis* Ducke	0.0	0.0	4.2
	Erythrina sp.	0.0	0.0	4.2
	Inga edulis Mart.	16.7	4.2	54.2
	Pterocarpus rohrii Vahl	6.3	2.1	0.0
	Pueraria montana (Lour.) Merr.	27.1	2.1	0.0
	Schizolobium amazonicum Ducke	2.1	0.0	6.3
Gesneriaceae	Gesneriaceae 1	8.3	0.0	0.0
Lamiaceae	*Vitex seudolia* L.	2.1	0.0	4.2
Lauraceae	*Nectandra cuspidata* Nees & Mart. ex Nees	2.1	0.0	0.0
	Ocotea sp.	0.0	0.0	6.3
Loranthaceae	*Phrygilanthus sp.*	2.1	0.0	0.0
Malvaceae	*Guazuma crinita* Lam.	4.2	0.0	8.3
	Theobroma cacao L.	8.3	16.7	0.0
Marantaceae	*Calathea sp.*	2.1	0.0	0.0
	Monotagma sp.	2.1	0.0	0.0
Meliaceae	*Cedrela odorata* L.	0.0	2.1	22.9
	Cedrela sp.	2.1	0.0	0.0
	Swietenia macrophylla King	0.0	0.0	6.3
Moraceae	*Ficus insipida* Willd.	0.0	0.0	4.2
	Moraceae 1	2.1	0.0	0.0
Musaceae	*Musa sp.*	8.3	0.0	41.7
Myrtaceae	*Psidium guajava* L.	0.0	0.0	4.2
Onagraceae	Onagraceae 1	2.1	0.0	0.0
Orchidaceae	Orchidaceae 1	2.1	0.0	0.0
Phyllanthaceae	*Phyllanthus niruri* L.	79.2	0.0	0.0
Phytolaccaceae	*Petiveria alliacea* L.	2.1	0.0	0.0
Piperaceae	*Peperomia sp.* 1	6.3	0.0	0.0
	Peperomia sp. 2	4.2	0.0	0.0
	Piper arcuatum Bl.	2.1	0.0	0.0
	Piper sp.	8.3	0.0	0.0
Poaceae	*Digitaria sp.*	10.4	0.0	0.0
	Echinochloa colona (L.) Link	2.1	0.0	0.0
	Paspalum conjugatum P.J.Bergius	4.2	0.0	0.0
	Paspalum sp. 1	2.1	0.0	0.0
	Paspalum sp. 2	4.2	0.0	0.0
	Paspalum sp. 3	2.1	0.0	0.0
	Paspalum sp. 4	2.1	0.0	0.0
	Rottboellia exaltata L.	12.5	6.3	0.0
Polygalaceae	*Polygala acuminata* Willd. ex Chodat	2.1	0.0	0.0
Polypodiaceae	*Microgramma sp.*	50.0	0.0	0.0
	Polypodium sp.	2.1	0.0	0.0
Pteridaceae	*Pityrogramma calomelanos* (L.) Link	2.1	0.0	0.0
Rubiaceae	*Capirona sp.*	2.1	0.0	10.4
	Coffea sp.	0.0	2.1	4.2

	Genipa americana L.	0.0	0.0	10.4
	Morinda citrifolia L.	0.0	0.0	4.2
	Palicourea sp.	2.1	0.0	0.0
	Simira sp.	4.2	0.0	0.0
	Rubiaceae 1	2.1	0.0	0.0
	Rubiaceae 2	2.1	0.0	0.0
Rutaceae	*Citrus sinensis* Osbeck	2.1	0.0	10.4
Sapotaceae	*Chrysophyllum cainito L.*	0.0	0.0	4.2
	Pouteria sapote (Jacq.) H.E.Moore & Stearn	6.3	0.0	4.2
Solanaceae	*Physalis angulata* L.	2.1	0.0	0.0
	Solanum sessiliflorum Dunal	6.3	4.2	0.0
	Solanum sp.	2.1	0.0	0.0
Urticaceae	*Cecropia palmata* Willd.	2.1	0.0	0.0
	Pourouma cacropiifolia Mart.	0.0	2.1	4.2
	Urtica sp.	16.7	0.0	0.0
	Urticaceae 1	2.1	0.0	0.0
Verbenaceae	*Verbena sp.*	2.1	0.0	0.0
	Lantana sp.	2.1	0.0	0.0
	Verbenaceae 1	4.2	0.0	0.0
-	Morphospecies 1	2.1	0.0	0.0
-	Morphospecies 2	2.1	0.0	0.0
-	Morphospecies 3	14.6	0.0	16.7

PUBLICATIONS OF THIS STUDY

Publications in peer reviewed journals

KIECK, J.S., ZUG, K.L.M., HUAMANÍ Y., H.A., GÓMEZ ALIAGA, R., CIERJACKS, A., 2016: Plant diversity effects on crop yield, pathogen incidence, and secondary metabolism on cacao farms in Peruvian Amazonia. Agric. Ecol. Environ. 222, 223–234.

Contributions to conferences

Runder Tisch Kakao, 2017

KIECK, J.S., ZUG, K.L.M., CIERJACKS, A.: The impact of cultivation conditions on the cacao seed quality. A study in Peruvian Amazonia. *(Poster)*

Choco Tec, 2016:

KIECK, J.S., ZUG, K.L.M., HUAMANÍ Y., H.A., GÓMEZ ALIAGA, R., CIERJACKS, A.: Influence of the cultivation conditions on the cacao seed quality–A case study from Peru. *(Talk)*

Runder Tisch Kakao, 2015

KIECK, J.S., GÓMEZ ALIAGA, R., LIEBEREI, R., CIERJACKS, A.: From coca to cocoa: Optimising the fermentation process of selected Trinitario fine and flavour clones in Peruvian Amazonia. *(Poster)*

KIECK, J.S., ZUG, K.L.M., CIERJACKS, A.: Are species composition and diversity related to pathogen incidence, yield and quality of cacao? A study in Peruvian Amazonia. *(Poster)*

Annual Conference of the Society for Tropical Ecology, 2015

KIECK, J.S., ZUG, K.L.M., CIERJACKS, A.: Are biodiversity, pest incidence, and environmental conditions related to the product quality of cacao? A case study from Peruvian Amazonia. *(Poster)*

Annual Conference of the Society for Tropical Ecology, 2014

KIECK, J.S., GÓMEZ ALIAGA, R., CIERJACKS, A., LIEBEREI, R.: From coca to cocoa: Economic sustainability of smallholder cocoa production by recovery of native cocoa varieties in Peru. (*Talk)*

Choco Tec, 2014

KIECK, J.S., GÓMEZ ALIAGA, R., LIEBEREI, R., CIERJACKS, A.: Development of a Standard Protocol for the Fermentation Process of Selected Trinitario Fine and Flavour Clones for Optimizing Product Quality and Economically Sustainable Cocoa Production in Peru. *(Poster)*

KIECK, J.S., ZUG, K.L.M., CIERJACKS, A.: Effects of cultivation conditions on the product quality of local cacao varieties and of the high-performance clone CCN-51 in Peru. *(Talk)*

Runder Tisch Kakao, 2013

KIECK, J.S., LIEBEREI, R.: Kakao als Alternatives Produkt: „Kakao statt Koka“. *(Poster)*

Choco Tec, 2012

KIECK, J.S., VEGA DELGADO, E., GÓMEZ ALIAGA, R., LIEBEREI, R.: Fermentation of Fine and Flavour cocoa. A case study of Peruvian varieties. *(Poster)*

ERKLÄRUNG ÜBER DEN PERSÖNLICHEN ANTEIL AN DEN PUBLIKATIONEN

1. PLANT DIVERSITY EFFECTS ON CROP YIELD, PATHOGEN INCIDENCE, AND SECONDARY METABOLISM ON CACAO FARMS IN PERUVIAN AMAZONIA

Veröffentlicht: KIECK, J.S., ZUG, K.L.M., HUAMANÍ Y., H.A., GÓMEZ ALIAGA, R., CIERJACKS, A., 2016: Plant diversity effects on crop yield, pathogen incidence, and secondary metabolism on cacao farms in Peruvian Amazonia. Agric. Ecol. Environ. 222, 223–234.

Planung des Projektes:	60%; in Zusammenarbeit mit Arne Cierjacks
Datenerhebung:	
Felderhebung:	70%; in Zusammenarbeit mit Katharina Zug und Roberto Gómez Aliaga
Bodenanalysen:	10%; in Zusammenarbeit mit Katharina Zug und Hugo Huamaní Yupanqui
Laboranalysen:	40%; in Zusammenarbeit mit Katharina Zug und Thomas Tumforde
Datenanalyse:	50%; in Zusammenarbeit mit Katharina Zug und Arne Cierjacks
Schriftliche Umsetzung:	70%**;** Korrekturen durch Arne Cierjacks

Die Planung des Projektes fand in Zusammenarbeit mit A. Cierjacks statt. Seine Erfahrung zur Planung des experimentellen Designs floss dabei mit meiner Erfahrung zu Kakao und der Situation vor Ort in Peru zusammen. Die Felddatenerhebung fand in Peru in Zusammenarbeit mit K. Zug und R. Gómez Aliaga (United Nations Office on Drugs and Crime, UNODC) statt. Die Auswahl der in Frage kommenden Parzellen lag dabei bei R. Gómez Aliaga, die Anpassung des Designs aufgrund der Durchführbarkeit sowie die Interviews der Kakaofarmer auf Spanisch bei mir und die praktische Felddatenerhebung bei K. Zug und mir zu gleichen Teilen. Die Voraufbereitung der Bodenproben erfolgte in Peru in Zusammenarbeit mit H. Huamaní Yupanqui durch K. Zug und mich. Die Aufbereitung und Vermessung der Bodenproben erfolgte an der UHH durch K. Zug. Die Aufbereitung der Proben für die Laboranalysen erfolgte zu gleichen Teilen durch K. Zug und mich, die Vermessung der Proben in der HPLC durch T. Tumforde. Die Aufbereitung der Rohdaten erfolgte zu gleichen Teilen durch K. Zug und mich, die statistische Berechnung erfolgte nach Anleitung von A. Cierjacks durch K. Zug und mich. Das Manuskript wurde von mir verfasst, mit umfangreichen Korrekturen durch A. Cierjacks.

2. INFLUENCE OF PULP PRE-CONDITIONING ON FERMENTATION AND RAW COCOA BEAN QUALITY IN TRINITARIO CLONES (*THEOBROMA CACAO* L.)

Vorbereitet für eine Veröffentlichung

Planung des Projektes: 80%; in Zusammenarbeit mit Reinhard Lieberei

Datenerhebung:

Versuchsdurchführung, Datenerhebung: 100% (Unterstützung durch die Mitarbeiter der UNODC, Tocache, Peru)

Verkostungsanalysen: 5%; Durchführung durch APPCACAO, Peru

Laboranalysen: 80%; in Zusammenarbeit mit Thomas Tumforde

Datenanalyse: 80%; in Zusammenarbeit mit Arne Cierjacks

Schriftliche Umsetzung: 80%; Korrekturen durch Arne Cierjacks und Bernward Bisping

Die Planung des Projektes erfolgte durch mich, R. Lieberei unterstützte mit seiner großen Erfahrung rund um Kakao. Versuchsdurchführung und Datenerhebung erfolgten durch mich in Peru. Die Mitarbeiter der UNODC organisierten die für die Versuche benötigten Kakaomengen von den Kakaobauern und unterstützten mich bei körperlich schweren Tätigkeiten. Kakaoproben wurden im Anschluss an die Versuchsdurchführung für die Verkostungsanalysen an APPCACAO (Asociación Peruana de Productores de Cacao) geschickt. Dabei bereitete ich die Proben vor. Die Ergebnisse wurden uns zur Verfügung gestellt. Die Aufarbeitung der Proben für die Laboranalysen erfolgte an der UHH durch mich, die Vermessung der Proben in der HPLC durch T. Tumforde. Die Aufbereitung der Rohdaten und die statistische Auswertung erfolgte durch mich, angeleitet von A. Cierjacks. Das Manuskript wurde durch mich verfasst und von A. Cierjacks und B. Bisping korrigiert.

3. FERMENTING IN A BOX: DO CACAO CLONE AND VESSEL WOOD TYPE MATTER?

Vorbereitet für eine Veröffentlichung

Planung des Projektes: 50%; in Zusammenarbeit mit Reinhard Lieberei, Daniel Kadow, Christina Rohsius und der UNODC, Peru

Datenerhebung:

Versuchsdurchführung, Datenerhebung: 100% (Unterstützung durch die Mitarbeiter der UNODC, Tocache, Peru)

Laboranalysen: 80%; in Zusammenarbeit mit Thomas Tumforde

Datenanalyse: 80%; in Zusammenarbeit mit Arne Cierjacks

Schriftliche Umsetzung: 80%**;** Korrekturen durch Arne Cierjacks und Bernward Bisping

Die theoretische Planung des Projektes in Bezug auf die Fermentation verschiedener Trinitario-Kakaoklone erfolgte hauptsächlich durch R. Lieberei, D. Kadow und C. Rohsius und wurde von mir vor Ort den Gegebenheiten angepasst. Die Planung der Versuche in Bezug auf die Auswirkungen der verschiedenen Holztypen auf die Fermentation erfolgte durch mich, nachdem meine Erfahrungen vor Ort in Peru einen möglichen Einfluss gezeigt hatten. Die Versuchsdurchführung und die Datenerhebung in Peru fanden durch mich statt. Die Mitarbeiter der UNODC organisierten den für die Versuche benötigten Kakao von den Kakaobauern und unterstützten mich bei körperlich schweren Tätigkeiten. Die Aufarbeitung der Proben für die Laboranalysen erfolgte an der UHH durch mich, die Vermessung der Proben in der HPLC durch T. Tumforde. Die Aufbereitung der Rohdaten und die statistische Auswertung erfolgte durch mich, angeleitet von A. Cierjacks. Das Manuskript wurde durch mich verfasst und von A. Cierjacks und B. Bisping korrigiert.

Bestätigung der Angaben durch die Unterschrift des Promotionsbetreuers:

Hamburg, 14.10.20

Ort, Datum

B. Bisping

Prof. Dr. Bernward Bisping

Prof. Dr. Bernward Bisping
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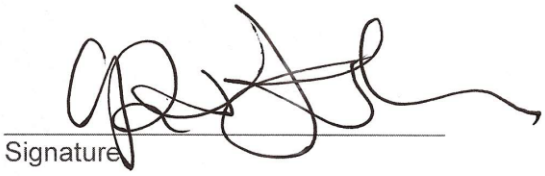
BESTÄTIGUNG ÜBER DIE KORREKTHEIT DER ENGLISCHEN SPRACHE

I, Gillian Stainforth, of 2/400 Beach Road, Beaumaris VIC 3193 Australia, (Australian Passport Number N4260677) confirm

that the thesis by Julia Susanne Cierjacks titled

"*Influence of farm management and fermentation on cocoa bean quality: Disentangling the driving factors of quality aspects in Theobroma cacao L. from Peruvian Amazonia*"

is written in comprehensible, understandable English.



Signature

24/10/2020

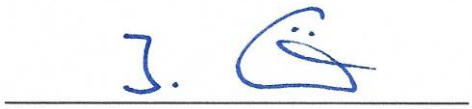
Date

EIDESSTATTLICHE VERSICHERUNG

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Dresden, 28.10.2020

Ort, Datum



Unterschrift