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Effects of mulch on soil pH and the growth of vanilla plants (*Vanilla planifolia*) in short-term greenhouse and agroforestry plantation experiments

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Abstract

Agroforestry produces various types of waste that can lower soil pH, increase the negative effect of allelochemicals and inhibit the growth of vanilla plants. Studies were conducted to examine the short-term effect (43 to 85 days) of dry and fresh mulches, compost and charcoal on soil pH and the growth of vanilla plants. Studies were performed in an agroforest and in a greenhouse using a completely randomised experimental design. The studies found that during rainy season in the agroforest, the addition of fresh grass clippings decreased soil pH by 0.15 relative control. In contrast, the same addition increased soil pH in greenhouse experiments by 0.22. The addition of compost also decreased soil pH in agroforestry by 0.15 but increased pH in greenhouse by 0.20. A similar effect was also found after the addition of charcoal. The addition of coconut husks and leaf litter in agroforest led to similar soil pH as the control, at 6.0 and 6.42 for coconut husks and leaf litter, respectively. High growth of vanilla plants occurred in a soil pH range from 6.0 to 6.5, irrespective of mulch materials added. This study indicates that environmental conditions change soil-mulch interactions, and that vanilla plants thrive well in soil pH ranging from 6.0 to 6.5 in most of the mulch types tested.

Keywords: Charcoal; compost; growth; mulches; soil pH; *Vanilla*

Abbreviations: C-compost; CCh-mixed compost and charcoal; Ch-charcoal; CH-coconut husk; Cof0-*Coffea* leaf (control); CofC-*Coffea* leaf-compost; CofCh-*Coffea* leaf-charcoal; G-grass clipping, G0-*Gliricidia* (control); GC-*Gliricidia*-compost; GCh-*Gliricidia*-charcoal; GCH-grass clipping-coconut husk; Int. 1-internode 1, Int. 2-internode 2, etc.; OCCh-leaf litter without compost and charcoal; OCChM-without compost, charcoal and mixed leaf litter; TS0-topsoil (control): Exp-experiment. TSC-topsoil-compost; TSCh-topsoil-charcoal.

Introduction

The most prominent constraint for vanilla plantations in rain-fed farmland is drought during the dry season (Hernandez-Hernandez, 2011). Therefore, the addition of mulch and continuous shading is crucial to maintain soil moisture. Whereas the addition of mulch minimises water loss from the soil (Chakraborty et al., 2008; Shaxson and Barber, 2003), continuous shading minimises light intensity, a condition required by vanilla plants (Díez et al., 2017; Exley, 2011), and indirectly also reduce evaporation. Although mulch improves soil moisture and provides the vanilla plant with a slow release of nutrients from organic materials (Chambers et al., 2019), mulch can also inhibit the growth of vanilla plants through the release of allelochemicals (Hickman et al., 2020). Thus, vanilla plantations in traditional agroforestry systems encounter possible constraints through both drought during the dry season and allelochemicals released from the mulch.

In traditional agroforestry in Madagascar, the tutor plant comes from one of several existing plant families, such as Fabaceae and Rubiaceae (Hending et al., 2020). The diverse plant species in this type of system can produce various sources of vegetative waste for mulching, such as the dry fallen leaves of various trees, grass and weed clippings, etc. This system promotes the accumulation of soil carbon, improving soil health and potentially increasing the sustainability of vanilla production (Chan, 2008; Martin et al., 2020; Watteyn et al., 2020). More importantly, leaf litter variability is positively correlated with the rate of release of important nutrients, suggesting higher stability of ecosystems properties where diversity is higher (Bonanomi et al., 2010).

Unlike traditional farming methods that sustain diversity (Hending et al., 2020), plantations designed for intensive vanilla production modify the existing plant community. In this technique, invasive plants, such as *Gliricidia sepium* (Kueffer and Vos, 2004) are introduced, mulch is prepared from dry branches and weeds are eliminated (Medina et al., 2009). Although this technique is highly efficient, it makes vanilla plants sensitive to biotic and abiotic stress, such as drought, temperature and disease (Watteyn et al., 2020). This sensitivity can subsequently limit the production period. For example, a commercial vanilla plantation in Mexico has an average lifespan of 6 years; 3 years in a vegetative state and 3 years in a generative phase (Fernández et al., 2018). After attaining top production, the vanilla plantation undergoes a lag period with no production for about 4-7 years (Adiputra, 2018). The introduction of invasive tutor plants likely contributes to the sensitivity of vanilla plants in intensive production techniques (Callaway and Ridenour, 2004).

Invasive plant species often release allelochemicals to reduce competition for nutrients (Hickman et al., 2020; Lorenzo et al., 2013). When in the presence of *G. sepium*, vanilla plants show reduced chlorophyll content (Martínez et al., 2007), which is most likely attributable to less nitrogen availability after soil nitrogen is taken up by *G. sepium* for the massive growth of its leaves. These deciduous leguminous trees shed their leaves during the dry season (Lima et al., 2006; Simons and Stewart, 1998), exposing vanilla plants to excessive light (Quiros, 2011). The leaf litter from the tutor plants then releases nutrients that the vanilla plant partly relies on, and allelochemicals that potentially inhibit the root growth of the vanilla plant (Bonanomi et al., 2011). Thus, the intensive vanilla plantation methods decrease plant diversity and may reduce soil health and thus sustainability of vanilla production. Therefore, as proposed by Hending et al. (2020), non-forested vanilla plantations should follow a more traditional forested approach. However, although diverse species presence in the agroforest system makes its ecosystem properties more stable (Bonanomi et al., 2010), it remains unclear whether changes in soil pH after accumulation of various vegetative wastes, affects the vegetative growth of vanilla plants.

Vanilla, as a shallow-rooted plant, is regarded as sensitive to the release of allelochemicals from the decomposition of plant material (Krishna and Mohan, 2017; Chalker-Scott, 2007). Therefore, to protect vanilla roots from allelochemical exposure, selective use of both mulch materials and tutor plants is required. Unfortunately, this selection is highly complicated since plant vegetation in tropical regions produces vastly different kinds of compounds (Jensen and Ehlers, 2010) that are regarded an allelochemicals. To promote the use of local tutor plants and plant materials for mulching, the toxic compounds released by the vegetative waste needs be neutralised. This neutralisation may include composting or the addition of other materials that reduce the impact of allelochemicals.

Composting of plant materials has been widely employed to enhance nutrient availability in soil and can also degrade the toxic compounds in plants materials. Ghaly et al. (2011) found that biodegradation via composting of phenolic compounds in creosote-treated wood waste reduced the concentration of the phenolic compounds. In the experiment, the authors mixed wood chips with fresh compost before loading them into a bioreactor. After 15 days in the bioreactor, the phenolic compound content decreased to approximately 26%. This finding suggests that mulch materials can be mixed with compost to reduce allelochemicals.

Other studies have reported that the toxicity of allelochemicals increases under conditions of lower soil pH (Bonanomi et al., 2006). Bonanomi et al. (2006) suggested that neutralisation of toxic compounds in the soil can be achieved by the addition of a material that increases soil pH. The most likely materials for this purpose are charcoal or ash, both of which have been reported to increase soil pH (Berek and Hue, 2016; Demeyer et al., 2001; Steiner et al., 2007). Therefore, compost or charcoal could reduce the impact of allelochemicals and promote the use of local resources for tutor plants or mulch material. If this method is effective, plant diversity can be maintained, which can then allow for sustained productivity in vanilla plantations. Accordingly, experiments were conducted to examine the effect of various mulch materials on soil pH dynamics and vegetative growth in vanilla plants.

Results

Experiment 1. Agroforest studies

In the first agroforest study, topsoil around vanilla plants mulched only with coconut husk (CH) showed a similar pH (6.0) to the control topsoil located 1 m outside the mulch. In contrast, topsoil around plants mulched with mixed coconut husks and fresh grass clippings (GCH) had a slightly lower soil pH = 5.85 (Table 1). The different levels of pH in CH, GCH and control groups were not statistically significant (p = 0.57).

The growth of vanilla plants, as represented by the production of internodes (Int.), was affected by the addition of GCH. The internode of vanilla vines located between leaves 3 and 4 from the apex, referred to as Int. 1, was still developing when grass clippings were added (Fig 1B). This Int. attained full expansion on day 17 after the application of grass clippings (Fig 2). At full expansion, the average length of this internode was 9.81 and 9.17 cm in CH and GCH plants, respectively (Table 2). Statistically, the difference between both treatments was not significant (p = 0.399). The internode that emerged after Int. 1, referred to as Int. 2, attained full expansion on day 24 (Fig 2) and had an average length at full expansion of 9.83 cm in CH plants and 9.06 cm in GCH plants (Table 2). Statistically, this difference was also not significant (p = 0.30). A similar result was observed for the internodes that emerged thereafter, i.e., Int. 3, Int. 4, and Int. 5. At full expansion, Int. 1-5 in GCH plants were shorter than the same internodes of CH plants. Except for Int. 4, the growth rate of internodes in CH plants was higher than the growth rate of GCH plants (Table 2). On day 59, Int. 1-5 had already fully expanded but Int. 6-10 were still developing (Fig 3). The growth rate of Int. 6 to Int. 10 of GCH plants were mostly lower than that of CH plants. After the addition of grass clipping for the second time, 30% of GCH plants showed tip burn symptoms (Fig 1D), while CH plants showed healthy growth (Fig 1C).

In the second agroforest study, the addition of leaf litter (0CCh) maintained soil pH for 29 days, but the addition of mixed leaf litter and compost (C) decreased soil pH in the same period (Fig 4). The initial soil pH (Day 1) was in the range of 5.9 to 6.40. The lowest pH was recorded in plants with mixed leaf litter-compost-charcoal (CCh) added and the highest pH was recorded in plants with only leaf litter (0CCh) added. Statistically, the initial soil pH did not different significantly between treatments (p = 0.322). On day 15, soil pH in 0CCh and Ch plants was unchanged at 6.4 and 6.1, respectively, but soil pH in CCh and 0CChM increased to 6.3 and 6.4, respectively, while the soil pH in C plants decreased from 6.3 to 6.2. On day 29, soil pH in 0CChM plants was further increased to 6.5 and soil pH in 0CCh plants was unchanged at 6.4. Soil pH in Ch and CCh plants decreased to 6.0 and in C plants, decreased to 5.9. Statistical analysis for the data collected from day 1 to day 41 showed that the average soil pH in C and Ch plants was significantly lower than in 0CCh (Table 3).

New leaves were present on vanilla plants 15 days after treatment. The highest number of new leaves per plant was recorded in CCh and 0CCh plants, followed by C and 0CChM plants, with the lowest number of new leaves recorded in Ch plants (Fig 5). On day 29, the highest growth was still shown by 0CCh plants, followed by CCh and C plants. The lowest growth was shown by Ch plants. Plotting the number of new leaves produced by vanilla plants in all treatments against soil pH reveals that the production of new leaves was high when the soil pH level was in the range of 6.0 to 6.5 and growth was lower when soil pH was less than 6.0 or higher than 6.5 (Fig 6). This confirms the previous findings in experiment 1 showing growth was reduced when soil pH was less than 6.0.

Experiment 2. Greenhouse study

During the dry season (June-September 2019) in the greenhouse experiment, the addition of CH led to similar soil pH as the control (6.01 and 6.03, respectively). However, the addition of grass clippings (G) led to a significantly higher soil pH of 6.23 (Table 1). These results are unsurprising as many agroforestry studies have shown the addition of fresh grass clipping decreases soil pH. Under the conditions of this experiment, growth of the root system in vanilla cuttings was delayed and the bud burst was slow. The cuttings began showing root growth on day 22 and, on day 49, the number of cuttings showing new root growth was 100%, 100% and 85% for CH, control, and G cuttings, respectively (Fig 7). In control plants, some new roots stopped growing after day 59. Unlike the production of new root systems, the production of new stem was very slow, especially in control and CH cuttings. Budburst was not seen in CH and control cuttings until day 84 after transplantation. Meanwhile, G cuttings showed bud burst, but only in 40% of transplanted cuttings (Fig 8).

In a separate experiment during the wet season (January to March 2020), vanilla plants in the G group had higher soil pH than those in the CH group; soil pH was 6.6, 6.5 and 6.5 for G, CH and GCH groups, respectively (Table 1). In this experiment, the emergence of a new root system occurred on day 13 after transplantation (Fig 9). In G and GCH cuttings, a high rate of new root production occurred from days 13–40, reaching 100% at day 40. However, CH cuttings had active growth only from days 13–33, reaching 70% growth. These CH cuttings did not increase growth until day 54 after transplantation (Fig 9). Budburst also occurred faster in G than CH cuttings; on day 54, the number of cuttings showing budburst was 40, 50 and 60% for CH, G and GCH cuttings, respectively (Fig 10). Thus, under greenhouse conditions, whether in the wet or dry season, the addition of G increased growth more than the addition of CH.

In a third experiment, the addition of fresh *Coffea* leaf, *G. sepium* leaf, compost and charcoal were considered during the wet season (December 2020 - January 2021) and revealed dynamic soil pH changes. In the control medium (TS0, Table 4), soil pH was stable at 6.5 for 41 days of observation. However, when mixed charcoal and fresh leaves (GCh or CofCh) were added, soil pH was increased at day 15, then returned to a pH similar to the control on day 41. The addition of mixed compost-fresh leaf (GC) also led to increasing soil pH, albeit at a slower rate, prior to becoming equal to the control at day 41. Without compost and charcoal (G0 and Cof0), the addition of fresh leaves did not lead to increasing pH except at day 26 for Cf0 (Table 4).

Under these conditions of changing soil pH, the production of the new root system was first observed on day 13 after transplantation. Root production was seen in cuttings grown with the addition of charcoal, whether mulched with fresh *Gliricidia* leaves (GCh, Fig 11a) or *Coffea* leaves (CofCh, Fig 11b), or not mulched (TSCh, Fig 11c). On day 41, cuttings grown with the addition of charcoal had attained 100% growth in GCh and TSCh. Cuttings grown with the addition of compost had delayed production of a new root system, on day 26. On day 41, growth attained was 80% in GC and CofC but 100% in TSC. Vanilla cuttings mulched with *Gliricidia* and *Coffea* leaves, without the addition of compost or charcoal (G0 and Cof0; Fig 11a, b), had a higher growth rates than control cuttings (TS0, Fig 11c) and attained 100% growth at day 41. This growth was also higher than vanilla cuttings with compost or charcoal added. Thus, under the condition of these experiments, production of new root systems in vanilla cuttings were enhanced by the addition of fresh *Gliricidia* and *Coffea* leaves.

In summary, these three greenhouse studies consistently found the addition of fresh vegetative mulch led to higher soil pH. This finding matched that of the field study, where the addition of fresh grass clippings, compost and charcoal decreased soil pH. A plot of root system production against soil pH, suggested growth was optimal in a soil pH range from 6.0-6.5 (Fig 12), in agreement with results from the agroforestry study.

Discussion

The sustainability of vanilla plantations in rain-fed farmland is dependent upon various factors, such as the sensitivity of vanilla plants to drought and allelochemicals (Bonanomi et al., 2011; Chalker-Scott, 2007; Hernandez-Hernandez, 2011). Therefore, minimising the affect of these detrimental factors is critical for maintaining growth and productivity of the plantation. For example, selective application of mulch on the vanilla plantation may minimise growth constraints for vanilla. However, the diversity of plant populations in the agroforest and the complexity of plant interactions through the soil make monitoring for a healthy soil that promotes optimal growth of vanilla plants complicated. For example, mulch material in the agroforest is available in high amounts and the application of mulch is crucial to inhibit water evaporation during the dry season. However, mulch material can contain allelochemicals and methods for alleviating the effect of such chemicals can not always be directly implemented in agroforest management. Thus, a simpler diagnostic method is required to monitor soil for the growth of vanilla plants in rain-fed agroforests. This study provides knowledge that can be applied to maintaining soil health.

The addition of fresh grass clippings decreased soil pH in the agroforestry study by 0.15 (agroforestry study 1, Table 1), but the same additions increased soil pH in the greenhouse study for 0.22 (greenhouse study 1, Table 1). These results reveal that mulch-soil interactions are modified by environmental conditions. In the greenhouse, pots were mounted 1 m above the soil surface, which led the rainwater to drain out quickly. The high porosity of the soil medium in pots also enables more air to enter the soil. This aeration then changes interactions between fresh mulches, such as grass clippings, and soil. In contrast, in the agroforest, rainwater is held under the mulches and less air enters the soil. Therefore, mulch-soil interactions are mostly aerobic in greenhouse and mostly anaerobic in agroforests. According to Bonanomi et al. (2006), the aerobic and anaerobic conditions in decomposition produce contrasting dynamics of phytotoxicity. Accordingly, the addition of fresh grass clipping in the agroforest during the wet season decreases growth (Table 2), but increases growth in the greenhouse (Figs 9, 10). A further interactive effect was seen when compost was added. In the greenhouse, this addition increased soil pH from 6.5 to 6.7 (Table 4), but in the agroforest, the pH instead decreased from 6.32 to 6.17

Table 1. Soil pH in agroforestry and in greenhouse study after addition of coconut husk and grass clippings. CH-vanilla plants mulched with coconut husk; GCH-vanilla plants mulched with mixed coconut husk and grass clipping; G-vanilla plants mulch with grass clippings. Mean with the same letters do not different significantly at p<0.05.

Treatment	pH	SD	Experiment	Season
Control	6.00^{a}	0.23	Agroforestry study 1	Wet
CH	6.00^{a}	0.40		
GCH	5.85^{a}	0.41		
Control	6.01^{a}	0.20	Greenhouse study 1	Dry
CH	6.03^{a}	0.21		
G	6.23^{b}	0.40		
CH	6.50^{a}	0.00	Greenhouse study 2	Wet
GCH	6.50^{a}	0.00		
G	6.60^{a}	0.21		

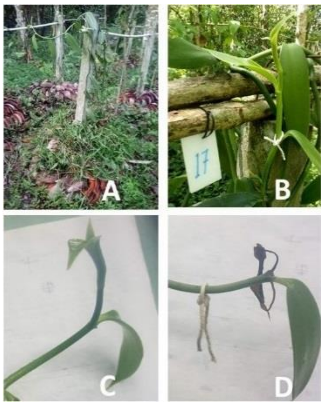

Fig 1. Agroforestry study to investigate the effect of fresh and dry mulch on soil pH and the growth of vanilla plants. A. Application of grass clipping and coconut husk in GCH plants, B. Tag was placed at internode located between L3 and L4 from the apex, C. Vanilla plants mulched only with coconut plants showed healthy growth, D. GCH plants showed tip burn symptom after added 8 litres fresh grass clipping for the second time.

Table 2. The average length of Int 1 to Int 10 observed at day 10, 17, 24, 30, 39 and 53 after the application of grass clipping on coconut husk mulch. CH-vanilla plants grown only with addition of coconut husk, GCH-vanilla plants grown with addition of coconut husk and grass clipping (GCH). Mean with the same letters do not different significantly at p<0.05.

Internode	CH			GCH		
	Length (cm)	SD	Growth rate (cm/day)	Length (cm)	SD	Growth rate (cm/day)
Int 1	9.81^{e}	2.56	0.11	9.17^{de}	2.38	0.07
Int 2	9.83^{e}	2.58	0.16	9.06^{de}	2.40	0.14
Int 3	9.01^{de}	3.22	0.28	8.46^{cde}	3.12	0.27
Int 4	8.26^{bcde}	4.15	0.25	7.25^{bcde}	4.00	0.26
Int 5	7.55^{bcde}	4.20	0.23	6.91^{bcde}	4.27	0.21
Int 6	6.26^{abcd}	4.78	0.24	5.54^{abc}	4.50	0.20
Int 7	6.05^{abc}	4.94	0.28	5.47^{abc}	4.87	0.25
Int 8	5.78^{abc}	4.92	0.31	5.40^{ab}	4.98	0.28
Int 9	5.31^{ab}	5.24	0.34	5.32^{ab}	4.99	0.32
Int 10	3.60^{a}	4.91	0.26	3.58^{a}	4.19	0.28

Table 3. Soil pH and production of new leaf in the second agroforestry study after the application of leaf litter (0CCh), mixed leaf litter with compost (C), charcoal (Ch), charcoal and compost (CCh) and control (0CChM).

Treatments	Amendments Compost (g/plant)	Charcoal (g/plant)	pH	SD	New leaf	SD
CCh	1000	100	6.05^{a}	0.39	0.95^{a}	1.09
Ch	0	100	6.07^{ab}	0.24	0.55^{a}	0.94
C	1000	0	6.17^{abc}	0.40	0.85^{a}	1.18
OCChM	0	0	6.32^{bc}	0.24	0.55^{a}	0.88
OCCh	0	0	6.42^{d}	0.18	1.45^{a}	1.34

Table 4. Greenhouse study to examine the effect of charcoal and compost on soil pH and the growth of vanilla cutting mulched with fresh leaf of *Gliricidia sepium* and coffee. C-Compost; Ch-Charcoal; Cof0-coffee leaf without compost and charcoal; CofC-coffee-compost; CofCh-coffee-charcoal; G0-gliricidia sepium without charcoal and compost; GC-gliricidia sepium-compost; GCh-Gliricidia sepium-charcoal; Treat-treatment; TS0-top soil without compost, charcoal and mulched; TSCh-top soil with charcoal; TSC-top soil with compost. Mean with the same letters do not different significantly at $p<0.05$.

Treat	Amendments		Day 0		Day 13		Day 26		Day 41	
	Ch (g)	C (g)	pH	SD	pH	SD	pH	SD	pH	SD
GCh	20	0	6.50^{a}	.61	7.00^{b}	.00	6.50^{a}	.00	6.50^{ab}	.00
GC	0	20	6.50^{a}	.00	6.70^{ab}	.27	6.60^{a}	.22	6.60^{a}	.22
G0	0	0	6.70^{a}	.27	6.60^{a}	.22	6.50^{a}	.00	6.50^{ab}	.00
CofCh	20	0	6.50^{a}	.00	6.70^{ab}	.27	6.60^{a}	.22	6.50^{ab}	.00
CofC	0	20	6.60^{a}	.22	6.60^{a}	.22	6.50^{a}	.00	6.50^{ab}	.00
Cof0	0	0	6.70^{a}	.27	6.60^{a}	.22	6.90^{b}	.22	6.50^{ab}	.00
TSCh	20	0	6.50^{a}	.61	6.50^{a}	.00	6.40^{a}	.22	6.20^{b}	.44
TSC	0	20	6.20^{a}	.27	6.50^{a}	.00	6.50^{a}	.00	6.50^{ab}	.00
TS0	0	0	6.50^{a}	.00	6.50^{a}	.00	6.50^{a}	.00	6.50^{ab}	.00

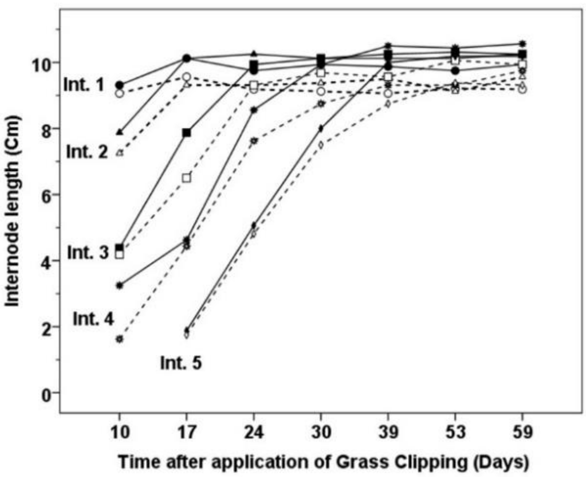

Fig 2. The growth of Int. 1 to Int. 5 in 2-3 years old vanilla plants after addition of CH and GCH mulch in the plantation —; CH plants; - - -, GCH plants.

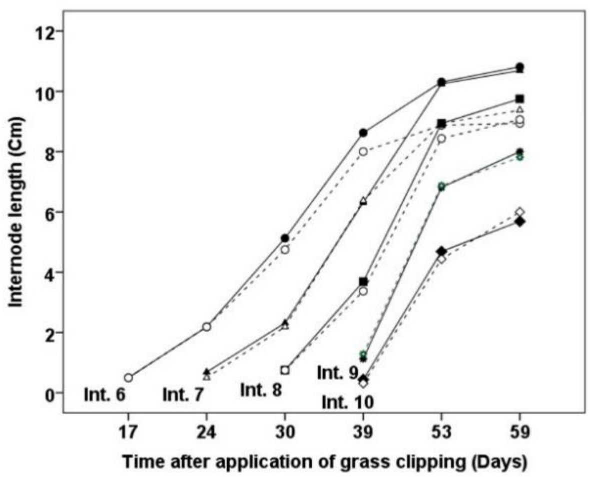

Fig 3. The growth of Int. 6 to Int. 10 in 2-3 years old vanilla plants after addition of CH and GCH mulch. Int 6-10 was still developing at the conclusion of the experiment. —, CH plants; - - -, GCH plants.

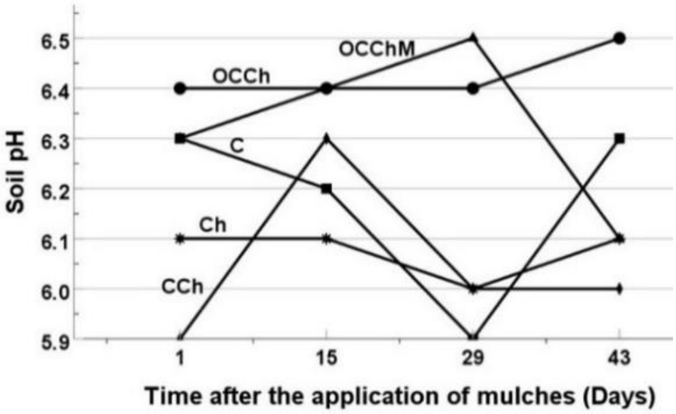

Fig 4. Soil pH in the second agroforestry study after the application of leaf litter (0CCh), mixed leaf litter with compost (C), charcoal (Ch), charcoal and compost (CCh) and control (0CChM).

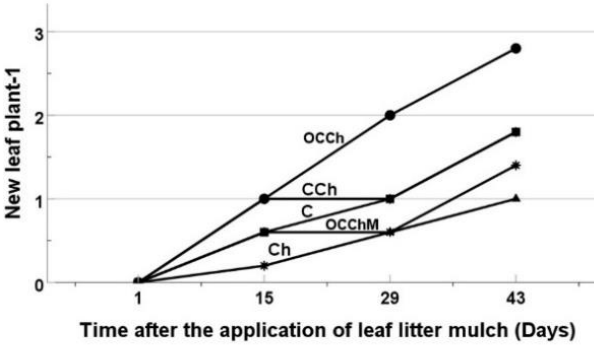

Fig 5. Production of new leaves in the second agroforestry study after the application of leaf litter (0CCh), mixed leaf litter with compost (C), charcoal (Ch), charcoal and compost (CCh) and control (0CChM).

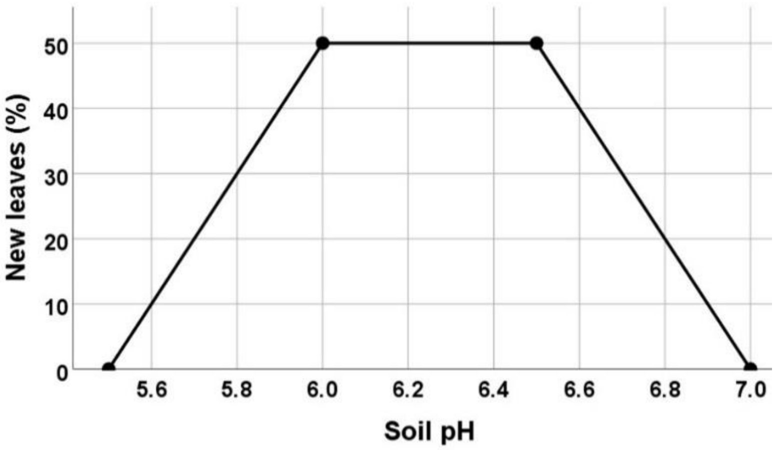

Fig 6. The total number of new leaves produced by 25 experimental plants during 41 Days period was 44. Most of the new leaves were produced by plants growing in soil pH level 6.0 and 6.5.

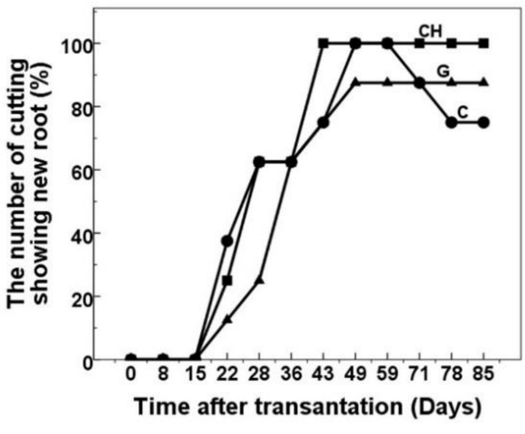

Fig 7. The production of the root system in vanilla cutting transplanted into growth medium with the addition of grass clipping and coconut husk mulch during the dry season in the greenhouse study.

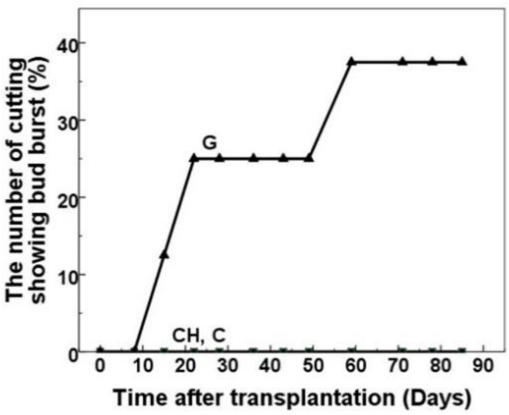

Fig 8. Production of the new stem in vanilla cutting transplanted into growth medium containing grass clipping and coconut husk during dry seasons in the greenhouse study.

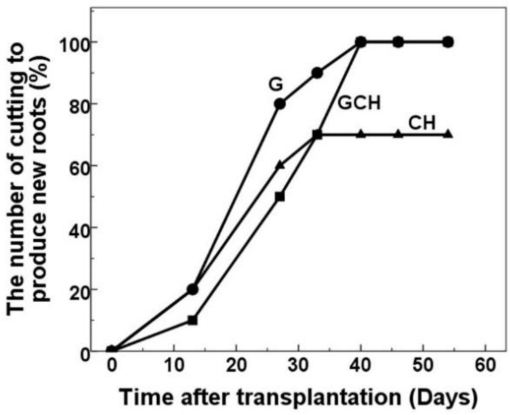

Fig 9. The production of the new root system in vanilla cuttings after transplantation into soil growth medium with the addition of G, CH and GCH mulches during the wet season.

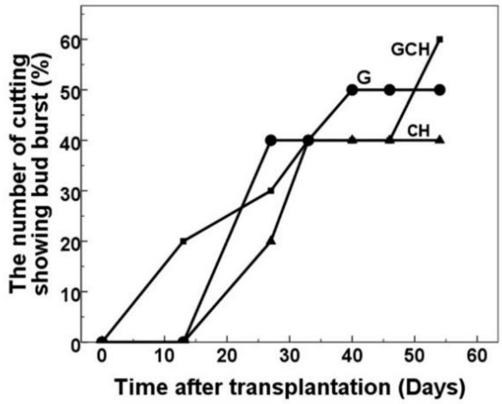

Fig 10. The production of the new stem (bud burst) in vanilla cuttings after transplantation into soil growth medium with addition of G, CH and GCH mulches during the wet season.

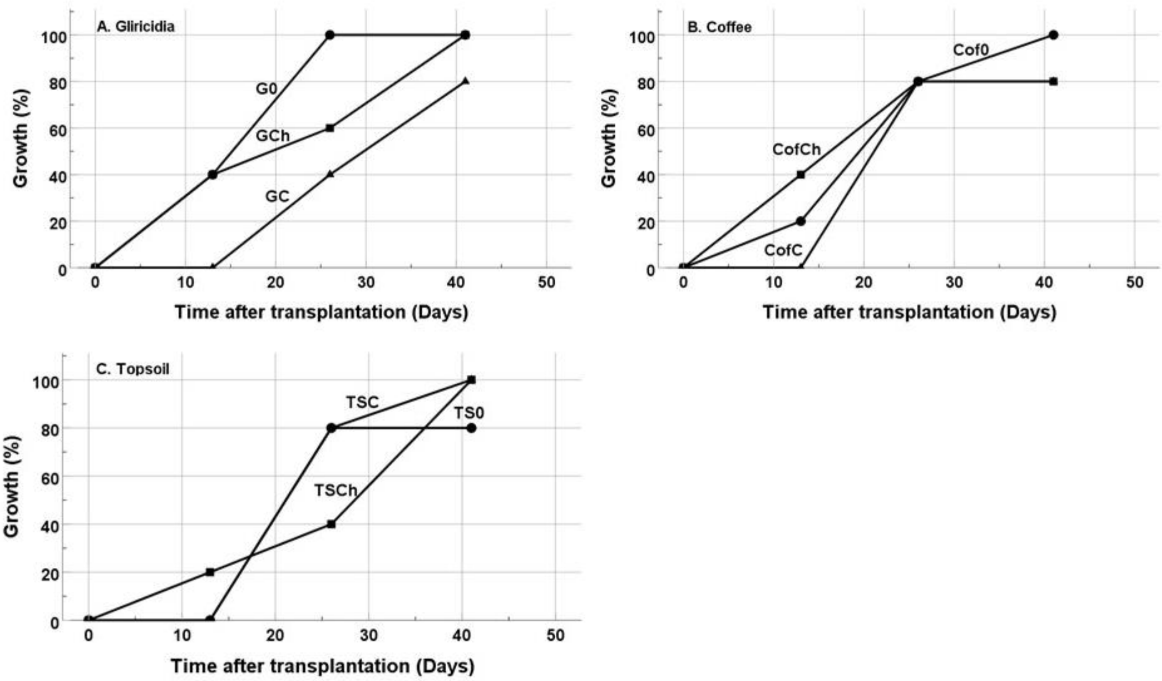

Fig 11. Production of the new root system in vanilla cutting mulched with A, fresh Gliricidia leaf; B. fresh Coffea leaf; C. control.

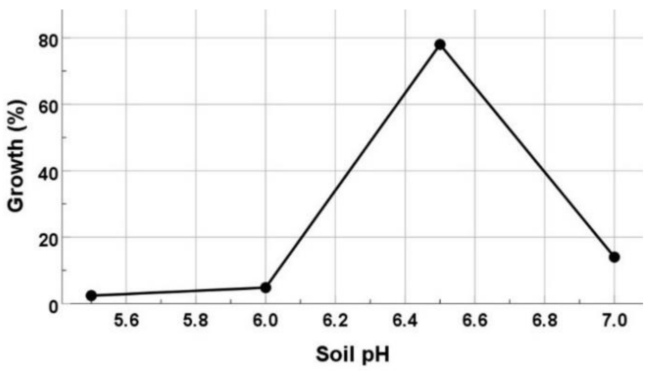

Fig 12. The total number of cutting to produce a new root system during the period of observation was 41 cuttings. The cuttings were growing in a growth medium with a range of soil pH level from 5.5 to 7 but the highest cutting to produce a new root system occurred in the growth medium with soil pH 6.5.

(Table 3). The different conditions in greenhouses and agroforests might affect not only phytotoxicity but also the activity of soil microbes. For example, nitrification, which converts ammonium to nitrite, results in the acidification of soil because the reaction produces H^+ ions (Sahrawat 2008). In the agroforest in the wet season, nitrification may be high and the addition of 100 g/plant charcoal was therefore unable to increase soil pH (Fig 4).

Unlike grass clippings, compost and charcoal, the addition of coconut husk and leaf litter did not decrease soil pH in either the agroforest or the greenhouse (Tables 1, 3). The most likely factor affecting this result is that the coconut husks and leaf litter contain a compound that can inhibit nitrification. If true, leaf litter in the agroforest would stabilise soil pH, making vanilla plantations in traditional agroforests more sustainable than under intensive plantation methods (Hending et al. 2020).

Under two experimental conditions, i.e., the greenhouse and agroforest, the growth of vanilla plants was high when soil pH was from 6.0 to 6.5 (Figs 6, 12). Results further indicated that vanilla plants thrive well in this soil pH range, irrespective of vegetative waste added for mulch. If this finding is robust, it has important implications in agricultural practices, particularly for the sustainability of vanilla plantations in diverse agroforests. The addition of a various types of vegetative mulch into the plantation appears to be safe for vanilla plants in so far as these additions do not affect soil pH beyond the optimal growing range.

Material and methods

*Plant materia*l

Vanilla plants established for 2-3 years were used in the agroforest 1 experiment and 5-month-old vanilla plants were used in the agroforest 2 experiment. Vanilla cuttings for greenhouse experiments were collected from other vanilla plants that had been growing on *Coffea* trees for more than 5 years.

D*ate and location of the experiments*

Experiments were conducted in an agroforestry plantation, defined as "land-use systems and technologies in which woody perennials (such as trees, shrubs, palms or bamboos) and agricultural crops or animals are deliberately grown on the same parcel of land in some form of spatial and temporal arrangement …" (FAO, 2019). As a land management unit, the plantation consists of various trees species, such as *Magnolia*, *Theobroma*, *Coffea*, *Artocarpus*, *Gliricidia*, *Michelia*, and *Dysoxylum*. For field experiments, vanilla plants used had been previously planted under the various shading tree species at various times before experimental mulches were applied. Thus, the age of the vanilla plants varied widely when the experiment commenced. The agroforestry study 1 was performed during the rainy season from 16 December 2019 until 15 March 2020 using a completely randomised experimental design with two treatments and 10 replicates. The agroforestry study 2 took place from 2 January 2021 until February 2021, using a similar experimental design with five treatments and five replicates. For the greenhouse studies, a simple shaded greenhouse was built on the plantation and three separate experiments were performed. The first experiment, using three treatments and eight replicates, was performed in the dry season from 8 June to 1 Sept. 2019, the second experiment with three treatments and 10 replicates was performed in the wet season from 19 January to 15 March 2020 and the third experiment was performed in the wet season from 21 Dec 2020 to 31 January 2021 using nine treatments and five replicates. All greenhouse experiments used completely randomised experimental designs. Experiments were conducted in the Tabanan regency at latitude 8º 23' 17.09" S and longitude 115º 6' 18.37" E at an elevation of 686 m.

Experiment 1. Agroforest study
Addition of dry coconut husk and fresh grass clipping mulches

Vanilla plants (2-3 years-old) that survived the long dry season in 2019 had thick coconut husk mulch added around their base during the wet season (starting 16 December 2019). The amount of coconut husk added per plant was equal to approximately 10-15 coconut fruit, which provided a mulch volume of approximately 25 cm high and 50 cm in diameter. Twenty vanilla plants labelled with numbers from 1 to 20 were used in this experiment.

Fresh grass clippings were then applied twice to odd-numbered vanilla plants. The first application took place on 25 December 2019 and the second application on 15 March 2020. Approximately 10-litre of fresh grass clippings collected from the local plantation were added to the plants, which are hereafter referred to as GCH plants (Fig 1A). Even-numbered plants had only coconut husk mulch applied and are referred to as CH plants. Just after the addition of fresh grass clippings, all experimental plants were tagged using a cotton rope to measure the effect of mulch on growth. Tags were placed on the internode located between leaf 3 and leaf 4 from the apex so as not limit growth. All leaves were actively growing; leaf 1 was just emerged, leaf 2 was partly unfolded, and leaves 3 and 4 were fully unfolded (Fig 1B). After the first application of grass mulch, growth of the vanilla vines was monitored every week by measuring the internode length. In this experiment, the tagged internode is referred to as internode 1 (Int. 1) and the internodes that emerged subsequently are referred to as Int. 2, Int. 3, and so on. The growth experiment continued for 2 months.

Soil pH in the rhizosphere of vanilla plants was measured on 24 February 2020 (61 days after the first application of grass clipping mulches) using a digital soil pH tester. Measurements were taken at three sites: the topsoil beneath coconut husk mulch in CH plants, topsoil beneath coconut husk in GCH plants and topsoil located 1 m outside the mulch area as a control.

Addition of leaf litter mulch, compost and charcoal

Agroforestry study 2 began on 2 January 2021 during the rainy season to examine the effect of compost, charcoal and dry leaf litter on the growth of vanilla plants. Vanilla plants that had been raised in a greenhouse for >3 months, were transplanted into an agroforest plantation consisting of various tree families, including Rubiaceae, Fabaceae, and Leguminosae. These newly transplanted vanilla plants were grown on *Coffea* trees and acclimatised for approximately 2 months before the application of compost, charcoal and mixed mulch. A total of 30 new experimental vanilla plants were grown, divided into five groups. Group treatments were as follows: Group 1 had 1 kg/plant of compost (C) added; Group 2 had 100 g/plant of charcoal (Ch) added; Group 3 had a mix of compost and charcoal (CCh) added at a ratio of 10:1; Group 4 had no compost or charcoal (0CCh) added; and Group 5 had no compost, charcoal or mulch (0CChM) added. Compost and charcoal were spread around the base of the vanilla vines to approximately 30 cm in diameter. All experimental vanilla plants except Group 5 were additionally covered with mixed leaf litter from various trees to a depth of 10 cm and 30 cm in diameter. Soil pH in the soil growth medium was measured every 2 weeks using a soil survey instrument. Grass and other weeds were cut manually, but their clippings were not removed. Just after the addition of the thick mixed leaf litter mulch, the vanilla plants were tagged using a cotton rope placed between leaves 3 and 4 from the apex. The growth of the vanilla vines was monitored every 2 weeks by counting the number of new leaves produced.

Experiment 2. Greenhouse study
Addition of coconut husk and fresh grass clipping mulches

The first greenhouse experiment began on 8 June 2019 during the dry season and was conducted in the same site as the field study. In this experiment, two nodes of vanilla cuttings were grown in five groups of pots, one cutting per pot with eight pots per group for a total of 40 cuttings. One upper leaf of these 2-node vanilla cuttings was left intact, but the lower leaf was excised and all root systems on the cutting were excised. The growth medium for these cuttings was 0.5 L topsoil collected from the local plantation and placed in 1-litre pots. The pots were then filled with mulch. The first group of pots was mulched with freshly mixed-grass clippings comprised mainly of grass and euphorbia weed (G). The second group were mulched with coconut husk (CH) and the third group acted as control cuttings (C) and had no mulch. Soil pH was measured using a soil survey instrument and the growth of cuttings observed every week.

Addition of fresh grass clippings, coconut husks and mixed coconut husk-grass clipping mulches

The second greenhouse experiment began 19 January 2020 during the wet season. In this experiment, vanilla cuttings were transplanted into three groups of pots containing a different ratio of topsoil, coconut husk and grass clippings. The first group of pots had 250 ml topsoil and 750 ml grass clippings (1:0:3) and are referred to as G cuttings. The second group of pots contained 250 ml topsoil and 750 ml of coconut husk chips (1:3:0) and are referred to as CH cuttings. The third group contained 250 ml topsoil, 375 ml coconut husk chips and 375 ml grass clippings (1:1.5:1.5) and are referred to as GCH cuttings. Each group consisted of 10 pots, with one cutting per pot, for a total of 30 pots in this experiment. Soil pH in this experiment was measured on 24 February 2020 (36 days after the application of grass clippings). The growth of the cuttings was monitored each week.

Addition of fresh *Coffea* leaf and *G. sepium* leaf mulches, compost and charcoal

In this experiment, the addition of deciduous *G. sepium* and evergreen *Coffea* leaf mulches were examined. Black plastic pots (1-litre) filled with 0.5 L topsoil, were divided into three groups. The first group had 20 g of charcoal added per pot, the second group had 20 g of compost added per pot and the third group acted as a control. Each of these groups were then further divided into three subgroups. The first subgroup was mulched with 20 g of deciduous *G. sepium* leaves, the second subgroup was mulched with 20 g of evergreen *Coffea* leaves and the third subgroup was not mulched, as a control. Each of the nine experimental units had five replicates. After addition of the mulches, 2-node vanilla vines were transplanted into the pots, with one cutting per pot (n = 45 cuttings) and all pots were placed inside the shaded greenhouse. Just after the transplantation, soil pH in each pot was measured using a soil survey instrument and the growth of the cuttings were subsequently measured every 2 weeks. This experiment was conducted during the wet season and transplantation was done on 21 December 2020 in Tabanan regency.

Statistical analysis

Data collected from this study were analysed using statistical software IBM SPSS Statistics version 25. The different effect on soil pH and growth after the application of grass clipping, coconut husk, leaf litters, compost, charcoal and fresh leaf were analysed using Univariate Analysis of Variance.

Conclusion

During the rainy season in the agroforest, the addition of grass clippings, compost and, to a lesser extend, charcoal decreased soil pH, but in the greenhouse these additions increased soil pH. Addition of coconut husk and leaf litter during the rainy season in the agroforest did not decrease soil pH. In the greenhouse, these additions also did not decrease soil pH. The growth of vanilla plants was high within a soil pH range from 6.0 to 6.5. The current study suggests that the addition of vegetative waste is safe for the growth of vanilla as long as it does not change soil pH beyond 6.0 to 6.5.

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