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Research Article

Phenol and Tannin Contents of Fresh Phyllodes and Leaf Litter Materials from Three *Acacia* Species in Brunei Darussalam

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ABSTRACT
Invasive *Acacia* species have been increasingly encroaching into degraded forests across Brunei Darussalam since their initial introduction in the 1990s. Understanding the secondary metabolites in these invasive species is crucial to assess their potential impact on leaf litter decomposition in invaded forest areas. This research focused on analysing the pH, total phenolic, and tannin levels of three invasive *Acacia* species (*Acacia auriculiformis* A. Cunn. ex Benth., *Acacia holosericea* A. Cunn. Ex G. Don, and *Acacia mangium* Willd.) and a native heath forest tree (*Buchanania arborescens* (Blume) Blume) found in Brunei. Measurements of pH, total phenolics, and tannins were taken and compared across these four species and between different leaf types (fresh phyllodes vs. leaf litter). Results showed that all invasive *Acacia* species exhibited higher pH, phenolic, and tannin contents than the native species. Fresh phyllodes generally had higher pH and phenolic content than leaf litter in all species, although tannin levels did not vary between fresh phyllodes and leaf litter samples. The elevated pH, phenolic, and tannin contents in the invasive *Acacia* species could make their leaves less palatable to herbivores and decomposers, potentially slowing decomposition compared to the native species, which could, in turn, affect decomposition rates in *Acacia*-invaded heath forests. Overall, these findings on species-specific and leaf type-specific variations in secondary compounds provide valuable insights into the decomposition patterns of *Acacia* species relative to native tree species.

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INTRODUCTION

In the early 1990s, invasive *Acacia*, originally from Australia, was introduced to Brunei Darussalam, Northwest Borneo. The purpose was to support the local timber and furniture industry and to help mitigate soil erosion caused by road constructions (Osunkoya et al. 2005). Four *Acacia* species are known in Brunei Darussalam: *Acacia mangium* Willd., *Acacia auriculiformis* A.Cunn. ex Benth., *Acacia cincinnata* F. Muell. and *Acacia holosericea* A.Cunn. ex G.Don (Sukri et al. 2017; Yusoff et al. 2019). Their ability to rapidly grow in nutrient -poor soils allows these *Acacia* species to effectively establish in disturbed and fire-affected coastal heath forests (Din et al. 2015; Tuah et al. 2020), further spreading inland into disturbed forests and displacing native vegetation (Osunkoya & Damit 2005).

Leaf secondary compounds are widely recognised as a key driver of plant leaf litter decomposability (Cornwell et al. 2008; Perez-Harguindeguy et al. 2013; de Oliveira et al. 2023), playing a major role in leaf litter decomposition and nutrient cycling (Chomel et al. 2016; Jaafar et al. 2022). They influence leaf litter decomposition directly through toxic effects limiting the growth and activity of decomposers (Lin et al. 2018). Phenolic compounds and tannins function as herbivory- and pathogen-defence for plants (Abdulrazak et al. 2000) and can negatively affect microbial decomposers (Rahman & Motiur 2012). Leaf tissue pH is an essential indicator of leaf degradation processes that correlates with tannins, as high tannin concentrations lower the pH in leaf tissue, reducing leaf digestibility to herbivores and detritivores (Perez-Harguindeguy et al. 2013).

Acacia phyllodes, barks, and fruits are the main sources of phenols and tannins for *Acacia* species (Rubanza et al. 2004; Sathya & Siddhuraju 2012; Anand & Mohan 2014; Elgailani & Ishak 2014, 2016; Amoussa et al. 2015; Arasaretnam & Venujah 2017; Ogawa & Yazaki 2018). In tropical Brunei Darussalam, several studies have attempted to quantify the antioxidant contents (including phenols; Sharifulazar 2017), fresh phyllodes and leaf litter pH of invasive *Acacia* species (Yusoff 2015; Jaafar et al. 2022), but investigations of total tannin content remain limited. Analysing phenols, tannins, and pH in invasive *Acacia* species' fresh phyllodes and leaf litter is crucial for understanding their decomposition in Brunei Darussalam's forests. These factors affect decomposition by influencing microbial activity and nutrient cycling. Thus, studying them offers insights into plant decomposition dynamics and ecological impacts. As such, this study aimed to quantify fresh phyllodes and leaf litter pH, total phenolic content (TPC), and total tannin content (TTC) in three invasive *Acacia* species (*A. auriculiformis*, *A. mangium* and *A. holosericea*) and one native heath forest species (*Buchanania arborescens* (Blume) Blume.) in Brunei Darussalam. Our research provides a unique contribution by examining the qualitative aspects, specifically TPC and TTC, of invasive *Acacia* species in Brunei. This novel perspective complements existing studies focused on environmental impacts, offering insight into the ecological implications of these species. Two hypotheses were formulated: (1) *Acacia* species fresh phyllodes and leaf litter will show higher pH, TPC and TTC than the native species due to their distinct physical properties and chemical composition, and (2) Fresh phyllode samples will exhibit higher pH, TPC, and TTC compared to leaf litter samples, due to the ongoing degradation process in leaf litter samples.

MATERIALS AND METHODS

Description of study site

The study site selected was located within the Universiti Brunei Darussalam (UBD) campus in the Brunei Muara district of Brunei Darussalam (4° 58'29.8"N, 114°53'36.6"E; Figure 1). This site was chosen due to the abundance of invasive *Acacia* species, with all four *Acacia* species (*A. auriculiformis*, *A. cincinnata*, *A. mangium* and *A. holosericea*) present around the campus area (Yusoff et al. 2019). UBD campus is located on the sandy soils of tropical heath or "Kerangas" forests (Tuah et al. 2020), which are acidic with low nutrient content (Sidiyasa 2001; Jaafar et al. 2016).

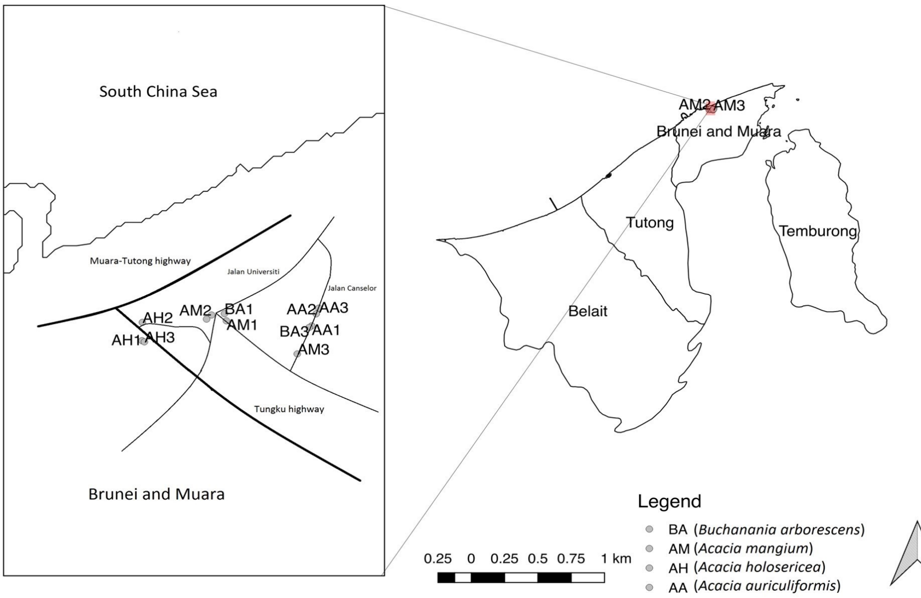

Figure 1. Location of the study site in remnant heath forests on Universiti Brunei Darussalam campus in Brunei Muara district. Three *Acacia* species (*A. mangium*, *A. holosericea*, and *A. auriculiformis*) and one native heath forest tree species (*B. arborescens*) were sampled at least 50 m apart from the same tree species. For each species, three trees were sampled.

Study species

Acacia mangium Willd.

In their natural habitat, *A. mangium* trees range from 7 to 30 m with a straight bole up to 4.5 m in height and a maximum diameter of 50 cm. The mature trees of *A. mangium* have pale grey-brown to brown coloured bark with rough and hard textures and fissures near the base, while the smooth dark green phyllodes are large and the length can be up to 27 cm and width of 3 to 10 cm with 3 to 5 main prominent nerves connecting at the base of the phyllodes near the lower margin (Krisnawati et al. 2011). *Acacia mangium* is closely related to *A. auriculiformis* and *A. holosericea* and is planted mainly for site rehabilitation due to its ability to grow well on infertile soils, particularly soils low in phosphorus (Francis 2002). It is a well-known plantation species due to its adaptability to grow on acidic soils with low nutrient availability, ability to shade out weeds efficiently, relative resilience towards diseases, and its desirable wood properties for use in various industries such as timber, furniture, and paper production (Krisnawati et al. 2011).

Acacia auriculiformis A. cunn ex Benth.

Trees of *A. auriculiformis* can reach a maximum height of 35 m, with long straight bole but most commonly are 8 to 10 m in height with a short, heavily branched trunk (Pinyopusarerk et al. 2018). Mature phyllodes are 8 to 20 cm long, with 3 to 6 pronounced parallel longitudinal nerves and a distinct node at the base of the phyllode (Pinyopusarerk et al. 2018). *Acacia auriculiformis* can withstand various conditions outside its natural habitat, such as coastal sands and waterlogged soils (Turnbull et al. 1998), highly alkaline, acidic, and saline soils, and a wider range of temperatures (Pinyopusarerk et al. 2018).

Acacia holosericea A. Cunn. Ex G. Don

Mature trees of *A. holosericea* can grow to an average height of less than 8 m with smooth bark (Orwa et al. 2009). The length of the thick phyllodes ranges between 10 to 25 cm long and typically 1.5 to 10 cm wide with three to five longitudinal veins (Orwa et al. 2009). In comparison to *A. mangium* and *A. auriculiformis*, *A. holosericea* tree can be distinguished by its phyllodes that are densely covered by fine hairs, giving an appearance of silvery green colour to the foliage (Liu et al. 2012; Yusoff 2015).

Buchanania arborescens (Blume) Blume

Buchanania arborescens is a native tropical tree species of the family Anacardiaceae that is commonly found on gentle slopes, terraces, raised beaches, or near seawater at 150 m altitude and also inhabits secondary forest as well as degraded and remnant heath forests of Brunei Darussalam (Coode et al. 1996). *Buchanania arborescens* tree can be recognised by its distinct, creamy white coloured flowers and shiny leaves that are oblong and narrowly obovate with upturned and wavy sides (Pesiu et al. 2016). This species was selected as a comparison to the three *Acacia* species due to its abundance and co-occurrence with *Acacia* species within the remnant heath forests in and around the UBD campus area (Tuah et al. 2020).

Leaf Sampling

Sampling was carried out in March 2018. Three individual trees from each species were randomly selected and sampled for fresh phyllodes (for *Acacia* species), fresh leaves (for *B. arborescens*), and leaf litter. For each tree, three replicates were collected, resulting in a total of 72 samples (*Acacia* species fresh phyllodes, n = 27 and native species fresh leaves, n = 9; *Acacia* species leaf litter, n = 27 and native species leaf litter, n = 9). Individual trees selected were at least 50 m apart from co-occurring individuals of the same species. The diameter breast height (dbh) and height for the *Acacia* trees sampled ranged from 28.04 to 167.64 cm and 1.68 to 10.67 m respectively, while dbh and height for *B. arborescens* trees sampled ranged from 27.43 to 28.96 cm and 6.10 to 7.65 m respectively. During the leaf sampling, branches and twigs were cut from the trees using secateurs and telescopic pruning shears. Leaves that were fully exposed to sunlight with little or no damage by herbivores were collected as fresh phyllodes samples. For leaf litter samples, only the freshly fallen leaf litter was collected below each sampled tree and replicated three times per tree.

Immediately after sampling, both fresh phyllodes and leaf litter samples were air-dried for three weeks at room temperature. Air-dried samples were then manually crushed by hand and further ground using a ball mill (Model MM400, Retsch, Germany). Ground samples were transferred into sealed zip-lock bags for the analysis of pH, TPC, and TTC.

Determination of pH, Total Phenolic and Total Tannin Content.

The pH of each ground fresh phyllodes and leaf litter samples were measured following Perez-Harguindeguy et al. (2013). Ground leaf powder was dissolved in 1:8 volume ratio of distilled water and thoroughly mixed in a rotary shaker for 1 hour. pH was then determined using calibrated pH meter (Hanna Instrument Ltd, UK). Total phenolic content (TPC) and total tannin content (TTC) were analysed for each sample using a modified Carnegie protocol (2011) by Ainsworth and Gillespie (2007) for phenols, and by Toth and Pavia (2001), and Makkar et al. (2007) for tannins. Ground samples of 0.20 g were extracted and homogenised using 95 % methanol and polyvinylpyrrolidone (PVP). The absorbance was measured at 750 nm of triplicate samples on a microplate reader (Biotek ELx800, USA). The calibration curve of gallic acid was plotted in the range of 25-200 mg L^{-1}. The TPC and TTC values were calculated from the linear regression of the calibration curve. Thus, TPC and TTC were expressed as gallic acid equivalents (GAE) in mg per g dry mass of sample (mg g^{-1}).

Statistical Analysis

All statistical analyses were conducted using R version 3.4.4 (R Core Team 2018). Variables of pH, TPC, and TTC were initially subjected to separate Two-Way Analysis of Variance (ANOVA) tests to determine differences between the four study species, between leaf types (fresh phyllodes or leaf litter samples), and species by leaf type interaction. The two-way ANOVA for all variables did not record any significant interactions between species and leaf type. Therefore, separate one-way ANOVA tests were conducted for each variable to determine between-species differences for pH, TPC, and TTC. Between-species differences were analysed using one-way ANOVA for fresh phyllodes samples only, and separately for leaf litter samples only. Assumptions of normality and equal variances were tested and were not violated. Differences were considered significant when $P < 0.05$. A pair-wise multiple comparison of means between species and between leaf types was conducted using Tukey's honest significant difference (Tukey's HSD) tests when the one-way ANOVA test revealed significant differences.

RESULTS

The two-way ANOVA revealed significant differences in mean pH, TPC and TTC ($P < 0.001$) between the four study species and significant differences between the fresh phyllodes and leaf litter sample types for pH and TPC ($P < 0.001$; Table 1). However, differences in TTC between fresh phyllodes and leaf litter samples were not significant ($P > 0.05$). No significant interactions were detected between species and leaf types for any of the three variables measured ($P > 0.05$; Table 1).

All *Acacia* species recorded significantly higher mean pH, TPC and TTC compared to the native species regardless of leaf type ($P < 0.05$; Figure 2; Table S1), and fresh phyllodes recorded significantly higher mean pH, TPC and TTC compared to the leaf litter samples regardless of species ($P < 0.05$; Figure 2; Table S2). For fresh phyllodes samples, the highest mean pH recorded was for *A. auriculiformis* (5.62 ± 0.12), while the lowest mean pH recorded was for *B. arborescens* (4.91 ± 0.08). For leaf litter samples, the highest mean pH was for *A. holosericea* (4.94 ± 0.07), while the lowest mean pH was for *B. arborescens* (4.31 ± 0.08; Figure 2).

All three *Acacia* species consistently recorded significantly higher mean TPC than the native species for fresh phyllodes samples ($P < 0.01$; Figure 2), with the highest mean TPC recorded in *A. holosericea* (25.33 ± 0.61 mg g^{-1}), while the lowest mean TPC was recorded in native species, *B. arborescens* (14.69 ± 2.00 mg g^{-1}). For leaf litter samples, mean TPC was significantly higher in all three *Acacia* species than native species, *B. arborescens* ($P < 0.05$; Figure 2). The highest mean TPC was detected in leaf litter samples of *A. holosericea* (19.80 ± 0.90 mg g^{-1}), while the lowest mean TPC was recorded in leaf litter samples of native species *B. arborescens* (9.80 ± 2.98 mg g^{-1}).

DISCUSSION

Variation in pH, Total Phenol Content (TPC) and Total Tannin Content (TTC) Between Species

This study recorded significant differences in the mean pH, TPC and TTC between the four species investigated. Regardless of the leaf types (either fresh phyllodes or leaf litter samples), all three *Acacia* species (*A. auriculiformis*, *A. holosericea*, and *A. mangium*) consistently showed significantly higher

Table 1. A two-way analysis of variance (ANOVA) for differences between species (*Acacia auriculiformis*, *Acacia holosericea*, *Acacia mangium* and *Buchanania arborescens*), and between leaf type (fresh phyllodes or leaf litter samples), for the following variables: (a) pH (b) total phenolic content (mg g^{-1}) and (c) total tannin content (mg g^{-1}). The degree of significance is indicated as follows: *P < 0.05; **P < 0.01; ***P < 0.001.

	(a) pH				(b) Total phenolic content (mg g^{-1})				(c) Total tannin content (mg g^{-1})			
Factors	df	MS	F-value	P	df	MS	F-value	P	df	MS	F-value	P
Species	3	0.508	13.391	<0.001 ***	3	112.380	18.639	<0.001 ***	3	10.539	21.625	<0.001 ***
Type	1	1.808	47.692	<0.001 ***	1	267.020	44.288	<0.001 ***	1	0.250	0.514	0.484
Species x Type	3	0.033	0.865	0.492	3	4.720	0.782	0.521	3	0.538	1.105	0.376

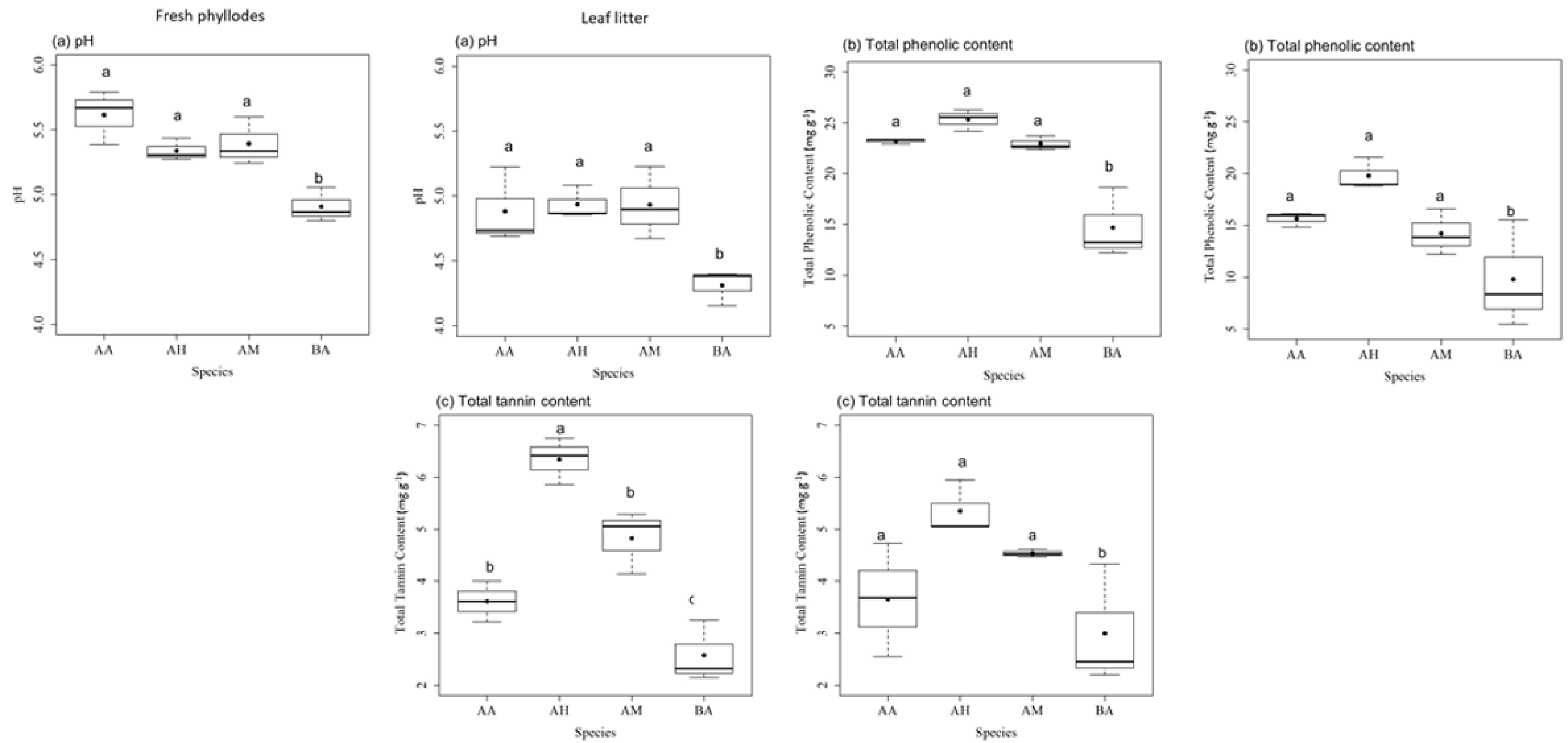

Figure 2. Variation in pH, total phenolic content (mg g^{-1}), and total tannin content (mg g^{-1}) between three *Acacia* species (*Acacia auriculiformis*, AA; *Acacia holosericea*, AH; *Acacia mangium*, AM) and native species (*Buchanania arborescens*, BA). Data values for pH (both fresh phyllodes and leaf litter) and total phenol content (fresh phyllodes) were log_{10} transformed due to a violation of its normality, but untransformed data were used in the presentation. Different letters within a panel indicate significant differences at P < 0.05 as obtained from Tukey's HSD after one-way analysis of variance (ANOVA). Closed circles represent mean values within each species.

mean pH, TPC and TTC than the native heath forest tree species, *B. arborescens*. This indicates that the invasive *Acacia* species studied contained higher concentrations of secondary compounds in their phyllodes compared to the leaves of this native heath forest species. This finding is consistent with higher initial leaf litter quality (N or C/N, lignin/N, tannin and phenols) of nitrogen-fixing trees than non-nitrogen-fixing trees in this case, *B. arborescens* (Gholami & Sayad 2022). Further, our results are consistent with previous studies on other *Acacia* species such as *Acacia ataxacantha*, *Acacia brevispica*, *Acacia confusa*, *Acacia nilotica*, *Acacia nubica*, *Acacia reficiens*, *Acacia senegal*, *Acacia seyal*, *Acacia sinuate* and *Acacia tortilis* that concluded these *Acacia* species were rich sources of phenols and tannins (Abdulrazak et al. 2000; Sathya & Siddhuraju 2012; Anand & Mohan 2014; Elgailani & Ishak 2014; Amoussa et al. 2015; Gupta & Bhat 2016; Arasaretnam & Venujah 2017).

Variation in TPC between all studied species may be largely due to genotypic factors that control the accumulation of polyphenolic compounds (Rubanza et al. 2004). Differences in leaf morphology can also influence TPC and TTC, for example, leaf thickness, leaf mass area, leaf age and environmental factors have been shown to positively correlate with TPC and TTC in providing leaf defense (Kitajima et al. 2012; Rahman et al. 2013). Despite being in the same genus, *A. holosericea* has a lower leaf mass area and leaf thickness than *A. mangium* and *A. auriculiformis* (Yusoff 2015). The lower leaf mass area and high leaf dry matter content of *B. arborescens* (Yusoff 2015) may influence their TPC and TTC levels.

Other studies outside Brunei Darussalam, using different plant parts of *Acacia* species, also reported inconsistent values of TPC (e.g. Abdulrazak et al. 2000; Sathya & Siddhuraju 2012; Anand & Mohan 2014; Amoussa et al. 2015). Low TPC value was only recorded by Anand and Mohan (2014) in methanol and aqueous extracts of leaves of *Acacia sinuate*. The TTC of *Acacia* species in this study was within the TTC range for fruits of *Acacia nubica*, *Acacia tortilis*, *Acacia brevispica* and leaves of *Acacia reficiens* and *Acacia senegal* (Abdulrazak et al. 2000). The difference in TPC and TTC of *Acacia* species between this study and other studies may be caused by the differences in climatic conditions and other environmental factors. Higher concentrations of polyphenols have been detected in the tissues of plants growing in the tropics compared to those in temperate regions (Coley 1983). Additionally, variations in extraction methods and solvent used could explain some differences in the TPC and TTC measured (Medini et al. 2014). The use of different *Acacia* plant parts at different maturity could further result in different values as TPC and TTC vary among plants, from one part to another, and in any one organ (Elgailani & Ishak 2014).

We found that *Acacia* leaves, irrespective of species, had higher pH than leaves of our native species, *B. arborescens*. Similarly, Yusoff (2015) reported higher mean pH values for leaves of invasive *Acacia* species (*A. auriculiformis*, *A. holosericea* and *A. mangium*) than native pioneer species (*Dillenia suffruticosa* (Griff. ex Hook. f. & Thompson) Martelli, *Ploiarium alternifolium* (Vahl) Melch. and *Melastoma malabathricum* L.) and tree species (*Symplocos polyandra* Brand, *B. arborescens*) and *Callophyllum soulatrri* Burm. f.) from disturbed coastal heath forests in Brunei. However, Jaafar et al. (2022) recorded a lower pH of *A. mangium* leaf litter in mixed-dipterocarp and heath forests, respectively, when compared to *Acacia* species in this study. Fresh phyllodes pH or leaf litter pH can vary greatly even among different species growing in the same soils (Perez-Harguindeguy et al. 2013), due to differences in the concentrations, movement, and interaction of various chemical compounds (Cornelissen et al. 2006). The low pH of *B. arborescens* leaves may act as a defence against herbivores' attack because low fresh phyllodes pH corresponds with poor digestibility and palatability (Cornelissen et al. 2006, 2011).

Low pH in leaves may result from high amounts of TPC (Khoo et al. 2014), organic acids and chemical defence compounds, such as tannins (Perez-Harguindeguy 2013), indicating that pH and secondary compounds TPC and TTC are inversely proportional. However, this contrasts with the present study where all three *Acacia* species recorded high pH values as well as high TPC and TTC in comparison to the native species. A possible reason for variation in pH, TPC, and TTC between *Acacia* species and native species may be due to differences in the growing stage of plants (Medini et al. 2014), leading to changes in the distribution or dynamics of secondary compounds during plant development and life cycle (Bano et al. 2003).

A previous study by Yusoff (2015) recorded significantly higher mean pH and specific leaf area (SLA) in the fresh phyllodes of *Acacia* species (*A. mangium* and *A. auriculifomis*) than in pioneer species (*D. suffruticosa*, *P. alternifolium* and *M. malabathricum*) and native species (*S. polyandra*, *B. arborescens* and *C. soulatrri*). While we did not directly measure the SLA of fresh phyllodes and leaf litter among different species in our research, our results imply a potential relationship between pH and SLA. Drawing a comparison with the findings of Yusoff (2015), our study suggests that this pH-SLA association might contribute to the variations in pH observed between fresh phyllodes and leaf litter samples of *Acacia* species and native species.

Variation in pH, Total Phenolic Content (TPC) and Total Tannin Content (TTC) Between Fresh Phyllodes and Leaf Litter Samples

This study recorded significantly higher mean pH and mean TPC in fresh phyllodes samples compared to leaf litter samples but did not find any significant differences in mean TTC between fresh phyllodes and leaf litter samples. Higher mean TPC in fresh phyllodes samples compared to leaf litter samples may be caused by the decomposition process in which organic matter is degraded and nutrients are released in inorganic forms (Rahman et al. 2013) through rapid leaching from leaf litter (Kuiters 1990). The rate of leaching of TPC is further enhanced by the presence of leaf-decomposing organisms on the leaf material (Kuiters 1990).

The higher mean pH in fresh phyllodes samples compared to leaf litter samples is comparable to those recorded by Yusoff (2015) for green leaves of *A. auriculiformis*, *A. holosericea*, and *A. mangium* and by Jaafar et al. (2022) on the leaf litter of *A. mangium*. Taken together, these studies consistently recorded higher mean pH values for all fresh phyllodes samples than leaf litter samples of *Acacia* species. The observed low pH values in leaf litter samples might be attributed to the leaching of acidic substances from vacuoles (Swift et al. 1979). Low leaf litter pH could potentially impact litter decomposition rates as highly acidic leaf litter has been linked to reduced digestibility by detritivores (Cornelissen et al. 2006, 2011).

The absence of significant variations in the mean TTC between fresh phyllodes and leaf litter samples, across all studied species, may indicate an adaptation by *Acacia* species for nutrient-poor soils. Plant communities rich in tannin often occur on soils that are acidic and poor in nutrients (Rahman et al. 2013). We suggest that TTC in both fresh phyllodes and leaf litter samples of all study species were similar as a response to the acidic and low-nutrient soil conditions of heath forests (Sidiyasa 2001; Din et al. 2015). Tannin has a mechanism that forms stable complexes with a plant protein to decrease its digestibility and to act as a toxin (Zucker 1983; Schofield et al. 2001; Rahman & Motiur 2012). Additionally, leaves with high initial TTC tend to have a slow decomposition (Valachovic et al. 2004; Rahman & Motiur 2012). Therefore, higher mean TTC in *Acacia* species could reduce the decomposition rate in leaf litter by forming protein-tannin complexes that slow down the activities of detritivores.

Significance of Study Findings to Acacia Invasion in Brunei's Heath Forests

The lowest TPC and TTC levels recorded in the native heath forest species (*B. arborescens*) compared to the three *Acacia* species may reflect different resource conservation strategies between native and invasive species. By having high TPC and TTC, *Acacia* species can invest more in tissue protection than growth (Reich et al. 1997; Leishman et al. 2007; Yusoff 2015). In contrast, the lower TPC and TTC for *B. arborescens* suggest that this native species may prefer a different approach than *Acacia* species through conserving resources. Differences in the investment of secondary compounds can give an advantage to invasive *Acacia* species over the native species as the former may possess either allelopathic, defensive, or antimicrobial compounds that are not present in native species (Cappuccino & Arnason 2006; Callaway et al. 2008; Weidenhamer & Callaway 2010).

Fresh phyllode's pH has the potential to influence carbon cycling processes such as herbivory, leaf litter decomposition, and mycorrhizal symbiosis (Cornelissen et al. 2006) because fresh phyllode pH may influence leaf litter acidity that could modify the pH of the soil organic matter (Grubb et al. 1969; Finzi et al. 1998). Low pH, however, may cause detrimental effects on plants (Long et al. 2017) by inhibiting the assimilation of CO_2 in some plant species and inducing oxidative stress in plants (Long et al. 2017). The higher mean pH recorded in *Acacia* fresh phyllodes and leaf litter samples compared to those in *B. arborescens* may indicate an adaptation of invasive *Acacia* species to maintain their survival in acidic environments (Ehrenfeld et al. 2001; Ehrenfeld 2004).

High lignin, TPC, and TTC in invasive *Acacia* species have led to slower decomposition rates when compared to native leaf litter (Devi & Prasad 1991; Drenovsky & Batten 2007; Godoy et al. 2010). This contrasts with Ehrenfeld (2003), Ashton et al. (2005), and Zhang et al. (2014) who found that exotic species exhibit faster decomposition rates and produce leaf litter that decomposes faster than native species. Suhaili (2017) also observed faster decomposition rates of *Acacia* leaf litter than mixed-heath leaf litter, regardless of forest type, while Jaafar et al. (2022) showed that leaf litter in non-invaded heath forests decomposed faster than in *Acacia*-invaded forest but leaf litter of *A. mangium* decomposed faster than mixed-native leaf litter. The presence of TPC in the leaf litter may affect the population of microbial decomposers (Harrison 1971) and delay microbial decomposition of plant leaf litter (Salusso 2000). Thus, low TPC in the leaf litter of three *Acacia* species and native species compared to the TPC in fresh phyllodes was probably caused by the release of phenolic compounds to the soil through leaching (Kuiters 1990) might increase the decomposition rate of the leaf litter. Condensed tannin serves as a deterrent to detritivores functioning as a toxin, rather than inhibiting digestion through protein precipitation (Rahman et al. 2013). Tannins can decrease the decomposition of plant leaf litter by forming complexes with proteins during leaf senescence (Kuiters 1990). Leaf litter decomposition is correlated to tannin concentration (Coq et al. 2010), where high tannin concentration in leaves resulted in slow leaf litter decomposability (Wantzen et al. 2002; Valachovic et al. 2004).

The higher TPC, TTC, and pH observed in *Acacia* species, the less potential leaf palatability to herbivores and detritivores. Hence *Acacia* leaf litter could have slower decomposition rates than native trees. By having slow decomposition rates, high TPC and TTC in *Acacia* species could inhibit the growth of native trees by forming thick layers of leaf litter, altering the habitat (Meira-Neto et al. 2017). Other than that, a thick accumulation of *Acacia* leaf litter could also affect the growth and germination of seeds of native species (Vaccaro et al. 2009).

CONCLUSION

This study has shown that pH, TPC and TTC were significantly higher in all three studied *Acacia* species than in native species. This is indicative of differences in strategies for survival, resource acquisition and defence between the invasive *Acacia* species and native species when they co-occur in the same heath forest. Our assessment of pH, TPC and TTC across different species and leaf types offers valuable insights into the decomposition rates of various *Acacia* species. This comprehensive understanding of nutrient cycling processes in *Acacia*-invaded heath forests not only enhances our ability to develop targeted strategies and management plans for *Acacia* invasion in Brunei Darussalam but also offers insights into the resilience of native species. By elucidating the interactions between invasive *Acacia* species and native flora, we can better conserve biodiversity and ecosystem functions while mitigating the ecological impacts of invasion.

AUTHOR CONTRIBUTION

S.J. undertook data collection, analysis, and manuscript composition. R.S.S. oversaw manuscript refinement, project supervision, and funding acquisition. F.M. contributed to manuscript enhancement and provided supervision. S.M.J. led research design, project supervision, and manuscript writing.

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CONFLICT OF INTEREST

All authors declare that no financial/personal interest or belief could affect their objectivity and that there exists no conflict.

REFERENCES

Abdulrazak, S.A. et al., 2000. Chemical composition, phenolic concentration and in vitro gas production characteristics of selected *Acacia* fruits and leaves. *Asian-Australasian Journal of Animal Sciences*, 13(7), pp.934–940. doi: 10.5713/ajas.2000.934.

Ainsworth, E.A. & Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–CIOCALTEU reagent. *Nature Protocols*, 2(4), pp.875–877. doi: 10.1038/nprot.2007.102.

Amoussa, A.M.O., Sanni, A. & Lagnika, L., 2015. Antioxidant activity and total phenolic, flavonoid, and flavanol contents of the barks extracts of *Acacia ataxacantha*. *Journal of Pharmacognosy and Phytochemistry*, 4, pp.172-178.

Anand, S.P. & Mohan, G., 2014. Antioxidant activity, phenol, and flavonoid contents of *Acacia sinuate* (Lourr) Merr. *Bioscience Discovery*, 5, pp.24-27.

Arasaretnam, S. & Venujah, K., 2017. Modification of tannin extracted from the bark of *Acacia auriculiformis* for the antibacterial activity and application of metal adsorption. *Ruhuna Journal of Science*, 8, pp.90-102. doi: 10.4038/rjs.v8i2.30

Ashton, I.W. et al., 2005. Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. *Ecological Applications*, 15(4), pp.1263-1272. doi: 10.1890/04-0741

Bano, M.J. et al., 2003. Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis* antioxidant activity. *Journal of Agricultural and Food Chemistry*, 51(15), pp.4247-4253. doi: 10.1021/jf0300745

Callaway, R.M. et al. 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*, 89(4), pp.1043-1055. doi: 10.1890/07-0370.1

Cappuccino, N. & Arnason, J.T., 2006. Novel chemistry of invasive exotic plants. *Biology Letters*, 2(2), pp.189-193. doi: 10.1098/rsbl.2005.0433

Carnegie Institution for Science, 2011. Spectranomics Protocol: Total Phenol and Tannin Determination. Protocol Document.

Chomel, M. et al., 2016. Plant secondary metabolites: a key driver to litter decomposition and soil nutrient cycling. *Journal of Ecology*, 104(6), pp.1527-1541. doi: 10.1111/1365-2745.12644

Coley, P.D., 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs*, 53(2), pp.209-233. doi: 10.2307/1942495

Coode, M.J.E. et al., 1996. A *Checklist of the Flowering Plants and Gymnosperms of Brunei Darussalam*. Brunei Darussalam: Ministry of Industry and Primary Resources.

Coq, S. et al., 2010. Interspecific in leaf litter tannins drive decomposition in a tropical rain forest of French Guiana. *Ecology*, 91(7), pp.2080-2091. doi: 10.1890/09-1076.1

Cornelissen, J.H.C. et al., 2006. Foliar pH as a new plant trait: can it explain variation in foliar chemistry and carbon cycling processes among subarctic plant species and types? *Oecologia*, 147, pp.315-326. doi: 10.1007/s00442-005-0269-z

Cornelissen, J.H.C. et al., 2011. Leaf pH as a plant trait: species-driven rather than soil-driven variation. *Functional Ecology*, 25(3), pp 449-455. doi: 10.1111/j.1365-2435.2010.01765.x

Cornwell, W.K. et al., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters*, 11(10), pp.1065-1071. doi: 10.1111/j.1461-0248.2008.01219.x

de Oliveira, V.S. et al., 2023. Physical traits are better drivers than secondary compounds for leaf litter decomposition in a tropical heath vegetation. *Flora*, 309, pp.152402. doi: 10.1016/j.flora.2023.152402

Devi, S.R. & Prasad, M.N.V., 1991. Tannins and related polyphenols from ten common *Acacia* species of India. *Bioresource Technology*, 36(2), pp.189-192. doi: 10.1016/0960-8524(91)90179-N

Din, H., Metali, F. & Sukri, R.S., 2015. Tree diversity and community composition of the Tutong White Sands, Brunei Darussalam: a rare tropical heath forest ecosystem. *International Journal of Ecology*, 2015(1), 807876. doi: 10.1155/2015/807876

Drenovsky, R.E. & Batten, K.M., 2007. Invasion by *Aegilops triuncialis* (barb goatgrass) slows carbon and nutrient cycling in serpentine grassland. *Biological Invasions*, 9, pp.107-116. doi: 10.1007/s10530-006-0007-4

Ehrenfeld, J.G., Kourtev, P. & Huang, W., 2001. Changes in soil functions following invasions of exotic understory plants in deciduous forests. *Ecological Applications*, 11(5), pp.1287-1300. doi: 10.1890/1051-0761(2001)011[1287:CISFFI]2.0.CO;2

Ehrenfeld, J.G., 2003. Effect of exotic plant invasion on soil nutrient cycling processes. *Ecosystems*, 6, pp.503-523. doi: 10.1007/s10021-002-0151-3

Ehrenfeld, J.G., 2004. Implications of invasive species for belowground community and nutrient processes. *Weed Technology*, 18(sp1), pp.1232-1235. doi: 10.1614/0890-037X(2004)018[1232:IOISFB]2.0.CO;2

Elgailani, I.E.H. & Ishak, C.Y., 2014. Determination of tannins of three common *Acacia* of Sudan. *Advances in Chemistry*, 2014(1), 192708. doi: 10.1155/2014/192708

Elgailani, I.E.H. & Ishak, C.Y., 2016. Methods for extraction and characterization of tannins from some *Acacia* species of Sudan. *Pakistan Journal of Analytical & Environmental Chemistry*, 17, pp.43-49. doi: 10.21743/pjaec/2016.06.007

Finzi, A.C., Canham, C.D. & van Breemen, N., 1998. Canopy tree-soil interactions within temperate forests: species effects on pH and cations. *Ecological Applications*, 8(2), pp.447–454. doi: 10.1890/1051-0761(1998)008[0447:CTSIWT]2.0.CO;2

Francis, J.K., 2002. *Acacia mangium* Willd. U.S. Department of Agriculture Forest Service: Tropical Seed Manual, Washington, U.S.A.

Gholami, S. & Sayad, E., 2022. Grouping nitrogen fixing trees into discrete functional groups based on litter decomposition rate does not make sense. *Environmental Resources Research*, 10(2), pp.301 – 310. doi: 10.22069/ijerr.2023.19323.1347

Godoy, O. et al. 2010. Leaf litter traits of invasive species slow down decomposition compared to Spanish natives: a broad phylogenetic comparison. *Oecologia*, 162, pp.781-790. doi: 10.1007/s00442-009-1512-9

Grubb, P.J., Green, H.E. & Merrifield, R.C.J., 1969. The ecology of chalk heath: its relevance to the calcifuge-calcicole and soil acidification problem. *Journal of Ecology*, 57(1), pp.175–211. doi: 10.2307/2258215

Gupta, A.K. & Bhat, J.L., 2016. GC-MS analysis of methanol extract of *Acacia nilotica* (L.) leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(2), pp.50-53. doi: 10.7439/ijpc.v6i2.3003

Harrison, A.F., 1971. The inhibitory effect of oak leaf litter tannins on the growth of fungi, in relation to litter decomposition. *Soil Biology & Biochemistry*, 3(3), pp.167-172. doi: 10.1016/0038-0717(71)90011-3

Jaafar, S., Sukri, R.S. & Proches, S., 2016. An investigation of soil physicochemical variables across different lowland forest ecosystems of Brunei Darussalam. *Malaysian Journal of Science*, 35(2), pp.148-166. doi: 10.22452/mjs.vol35no2.6

Jaafar, S. et al., 2022. Differential impacts of *Acacia* invasion on nutrient fluxes in two distinct Bornean lowland tropical rain forests. *Forests*, 13(12), 2101. doi: 10.3390/f13122101

Khoo, L.T. et al., 2014. Bioassay-guided fractionation of *Melastoma malabathricum* Linn. leaf solid phase extraction fraction and its anticoagulant activity. *Molecules*, 20(3), pp.3697-3715. doi: 10.3390/molecules20033697

Kitajima, K. et al., 2012. How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species. *New Phytologist*, 195(3), pp.640-652. doi: 10.1111/j.1469-8137.2012.04203.x

Krisnawati, H., Kallio, M. & Kanninen, M., 2011. *Acacia mangium* Willd.*: Ecology, silviculture, and productivity.* Center for International Forestry Research, Bogor, Indonesia. doi: 10.17528/cifor/003392

Kuiters, A.T., 1990. Role of phenolic substances from decomposing litter in plant-soil interactions. *Acta Botanica Neerlandica*, 39(4), pp.329-348. doi: 10.1111/j.1438-8677.1990.tb01412.x

Leishman, M.R. et al., 2007. Leaf trait relationships of native and invasive plants: community – and global-scale comparisons. *New Phytologist*, 176 (3), pp.635-643. doi: 10.1111/j.1469-8137.2007.02189.x

Lin, H. et al., 2018. Secondary compounds of *Pinus massoniana* alters decomposers effects on *Quercus variabilis* litter decomposition. *Ecology and Evolution*, 8(18), pp.9439-9450. doi: 10.1002/ece3.4433

Liu, J. et al. 2012. Effects of three different nano-silver formulations on cut *Acacia holosericea* vase life. *Postharvest Biology and Technology*, 66, pp.8-15. doi: 10.1016/j.postharvbio.2011.11.005

Long, A. et al., 2017. Effects of low pH on photosynthesis, related physiological parameters and nutrient profiles of citrus. *Frontiers in Plant Science*, 8, 185. doi: 10.3389/fpls.2017.00185

Makkar, H.P.S., Francis, G. & Becker, K., 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animals*, 1(9), pp.1371–1391. doi: 10.1017/S1751731107000298

Medini, F. et al., 2014. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the *plant Limonium delicatulum. Journal of Taibah University for Science*, 8(3), pp.216-224. doi: 10.1016/j.jtusci.2014.01.003

Meira-Neto, J.A.A. et al., 2017. Early *Acacia* invasion in a sandy ecosystem enables shading mediated by soil, leaf nitrogen and facilitation. *Biological Invasions*, 20, pp.1567-1575. doi: 10.1007/s10530-017-1647-2

Ogawa, S. & Yazaki, Y., 2018. Tannins from *Acacia mearnsii* De Wild. Bark: tannin determination and biological activities. *Molecules*, 23(4), 837. doi: 10.3390/molecules23040837

Orwa, C. et al., 2009. Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

Osunkoya, O.O. & Damit, N., 2005. Population dynamics of the invasive *Acacia* species in Brunei Darussalam using matrix modeling. *Journal of Physical Science*, 16, pp.115-126.

Osunkoya, O.O., Othman, F.E. & Kahar, R.S. 2005. Growth and competition between seedlings of an invasive plantation tree, *Acacia mangium*, and those of a native Borneo heath-forest species, *Melastoma beccarianum. Ecological Research*, 20(2), pp.205-214. doi: 10.1007/s11284-004-0027-4

Perez-Harguindeguy, N. et al., 2013. New handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61, pp.167-234. doi: 10.1071/BT12225

Pesiu, E. et al., 2016. Tree species composition in Pulau Bidong and Pulau Redang. *Journal of Sustainability Science and Management*, 1, pp.48-60.

Pinyopusarerk, K. Midgley, S. & Thompson, L., 2018. *Acacia auriculiformis.* In *Trees for life in Oceania: conservation and utilisation of genetic diversity*. Canberra, Australia: Australian Centre for International Agricultural Research.

R Core Team, 2018. 'R: A language and environment for statistical computing', in *The R Project for Statistical Computing*, viewed from https://www.R-project.org

Rahman, M.M. & Motiur, R.M., 2012. Quantitative chemical defense traits, litter decomposition and forest ecosystem functioning. In Forest Ecosystems - More than Just Trees. IntechOpen, pp.295-314.

Rahman, M.M., et al., 2013. The role of quantitative trait of leaf decomposition and nutrient cycling of the forest ecosystems. *Journal of Forest Science*, 29(1), pp.38-48. doi: 10.7747/JFS.2013.29.1.38

Reich, P.B., Walters, M.B. & Ellsworth, D.S., 1997. From tropics to tundra: global convergence in plant functioning. *PNAS*, 94(25), pp.13730–13734. doi: 10.1073/pnas.94.25.13730

Rubanza, C.D.K. et al., 2004. Polyphenolics and tannins effects on in vitro digestibility of selected *Acacia* species leaves. *Animal Feed Science and Technology*, 119(1-2), pp.129-142. doi: 10.1016/j.anifeedsci.2004.12.004

Salusso, M.M., 2000. Biodegradation of subtropical forest woods from northwest Argentina by *Pleurotus laciniatocrenatus. New Zealand Journal of Botany*, 38(4), pp.721-724. doi: 10.1080/0028825X.2000.9512712

Sathya, A. & Siddhuraju, P., 2012. Role of phenolics as antioxidants, biomolecule protectors and as anti-diabetic factors- Evaluation on bark and empty pods of *Acacia auriculiformis*. *Asian Pacific Journal of Tropical Medicine*, 5(10), pp.757-765. doi: 10.1016/S1995-7645(12)60139-4

Schofield, P., Mbugua, D.M. & Pell, A.N., 2001. Analysis of condensed tannins: a review. *Animal Feed Science and Technology*, 91(1-2), pp.21-40. doi: 10.1016/S0377-8401(01)00228-0

Sharifulazar, D.S.H., 2017. *Effects of Acacia mangium extract on the fruit fly, Drosophila melanogaster*. Universiti Brunei Darussalam.

Sidiyasa, K., 2001. *Tree diversity in the rain forest of Kalimantan: The balance between biodiversity conservation and sustainable use of tropical rain forests*. Netherlands: The Tropenbos Foundation.

Suhaili, A.L.R., 2017. *The impacts of invasive Acacia mangium* Willd. *on litterfall production and decomposition in tropical heath forest habitats of Brunei Darussalam*. Universiti Brunei Darussalam.

Sukri, R. et al., 2017. Global Register of Introduced and Invasive Species - Brunei Darussalam (v2.4). Invasive Species Specialist Group ISSG. Dataset/Checklist. doi: 10.15468/ljnym4

Swift, M.J., Heal, O.W. & Anderson, J.M., 1979. *Decomposition in terrestrial ecosystem*. Oxford: Blackwell Scientific Publication.

Toth, G.B. & Pavia, H., 2001. Removal of dissolved brown algal phlorotannins using insoluble polyvinylpolypyrrolidone (PVPP). *Journal of Chemical Ecology*, 27, pp.1899-1910. doi: 10.1023/A:1010421128190

Tuah, W. et al., 2020. Post-fire impacts on tree diversity in coastal heath forests of Brunei Darussalam. *Scientia Bruneiana*, 19, pp.19-32. doi: 10.46537/scibru.v19i1.109

Turnbull, J.W., Midgley, S.J. & Cossalter, C., 1998. Tropical acacias planted in Asia: an overview. *ACIAR Proceedings*, 82, pp.14-28.

Vaccaro, L.E., Bedford, B.L. & Johnston, C.A., 2009. Litter accumulation promotes dominance of invasive species of cattails (*Typha* spp.) in Lake Ontario wetlands. *Wetlands*, 29, pp.1036-1048. doi: 10.1672/08-28.1

Valachovic, Y.S. et al., 2004. Leaf litter chemistry controls on decomposition of Pacific Northwest trees and woody shrubs. *Canadian Journal of Forest Research*, 34(10), pp.2131-2147. doi: 10.1139/x04-089

Wantzen, K.M., et al., 2002. How do plant-herbivore interactions of trees influence coarse detritus processing by shredders in aquatic ecosystems of different latitudes? *International Association of Theoretical and Applied Limnology*, 28(2), pp.815-821. doi: 10.1080/03680770.2001.11901827

Weidenhamer, J.D., & Callaway, R.M., 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. *Journal of Chemical Ecology*, 36, pp.59-69. doi: 10.1007/s10886-009-9735-0

Yusoff, A., 2015. *The effects of Acacia invasion on leaf litter nutrient and soil physicochemical properties of coastal Kerangas (heath) forest in Brunei Darussalam*. Universiti Brunei Darussalam.

Yusoff, A. et al., 2019. Effects of *Acacia* invasion on leaf litter nutrient and soil properties of coastal *Kerangas* forests in Brunei Darussalam. *Scientia Bruneiana*, 18(1), pp.1-10. doi: 10.46537/scibru.v18i1.87

Zhang, L. et al., 2014. Non-native plant litter enhances soil carbon dioxide emissions in an invaded annual grassland. *PloS ONE*, 9(3), e92301. doi: 10.1371/journal.pone.0092301

Zucker, W.V., 1983. Tannins: does structure determine function? An ecological perspective. *American Naturalist*, 121(3), pp.335-365. doi: 10.1086/284065

APPECNDICES

Table S1. Mean values of pH, total phenolic content (mg g^{-1}) and total tannin content (mg g^{-1}) between species, regardless of leaf type. Values are means ± standard error, SE. The mean values presented in this table were calculated averaging nine samples per species.

	Acacia auriculiformis	*Acacia mangium*	*Acacia holosericea*	*Buchanania arborescens*
pH	5.25 ± 0.10	5.16 ± 0.07	5.14 ± 0.06	4.61 ± 0.11
TPC (mg g^{-1})	17.4 ± 0.95	16.9 ± 1.03	20.4 ± 0.82	10.8 ± 1.21
TTC (mg g^{-1})	3.28 ± 0.23	4.27 ± 0.19	5.47 ± 0.17	2.79 ± 0.26

Table S2. Mean values of pH, total phenolic content (mg g^{-1}) and total tannin content (mg g^{-1}) between leaf type, regardless of species. Values are means ± standard error, SE. The mean values presented in this table were calculated averaging 36 samples per leaf type.

	Fresh phyllodes	Leaf litter
pH	5.31 ± 0.05	4.77 ± 0.07
TPC (mg g^{-1})	19.4 ± 0.73	13.4 ± 0.81
TTC (mg g^{-1})	4.04 ± 0.25	3.86 ± 0.20