The preliminary findings of appropriate soil conditions for growth and development of *Adenosma bracteosum* Bonati in Lo Go – Xa Mat National Park, Vietnam

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Abstract
The study was conducted to determine appropriate soil properties for Adenosma bracteosum Bonati via the coupling between the plant growth and soil conditions in Lo Go – Xa Mat National Park, Tay Ninh province, Vietnam. Experimental plots were installed in three grasslands based on the presence of A. bracteosum. The plant heights and number of individuals were measured monthly during the growing season for growth evaluation and soil samples were collected to determine the discrepancies in soil physicochemical properties between the chosen sites.

The results implied that A. bracteosum could not grow in soils with humidity < 18.4%, dry bulk density > 1.5 g.cm^{-3}, pH < 4.9, fine grain proportion > 54.2%, total nitrogen content < 0.1% and available phosphate content < 0.0113 mg.g^{-1}. The soil conditions in the habitats of A. bracteosum were probably just sufficient to maintain the appearance but not optimal for the plant growth and development.

Keywords: *Adenosma bracteosum,* Soil conditions, Grassland, Plant, Lo Go – Xa Mat National Park.

Introduction

Adenosma bracteosum Bonati is a medicinal plant commonly used in Vietnamese traditional medicine. The decoctions of *A. bracteosum* could effectively treat colds, hepatitis, polio, rheumatism and stomach ache. Additionally, several studies showed that the antioxidant components of their extracts might help to lower blood sugar levels and inhibit the growth of cancer cells in the human liver and lungs. The extracts of *A. bracteosum* were promising for preparing and producing drugs to treat gout, to protect the liver and to lower blood sugar.[2,8-10,14,20,25]

In Vietnam, *A. bracteosum* is not a rare medicinal species, but the distribution is limited, mainly concentrated in Kontum and Tay Ninh provinces[15]. In the Lo Go – Xa Mat National Park, *A. bractesoum* was mainly distributed in grasslands[15]. Thus, it was possible to create specific products from this herb to bring economic benefits to the National Park in particular and the Tay Ninh province in general.

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Nevertheless, the size of the *A. bracteosum* population in the National Park has decreased in recent years. In order to recover and increase the distribution area in the National Park or plant them to provide stable sources for production, understanding the ecological needs of *A. bracteosum* is essential. However, there was little research on the effects of environmental factors on plant growth and development.

Previous studies mainly focused on its chemical composition and the ability to detoxify and cure illnesses based on the essential oil ingredients of the plant[4,8-10,20,24,25]. Therefore, this study aimed to determine appropriate soil properties for *A. bracteosum* via the coupling between plant growth and soil conditions. Due to the various pedologic requirements, discrepancies in soil physicochemical properties between chosen areas could imply suitable soil properties for this species. The study would provide a reliable scientific basis to expand the distribution area of *A. bracteosum* in the park and to improve its productivity in cultivation.

Material and Methods

Study areas: Lo Go – Xa Mat National Park is located in the northwest of Tay Ninh province, Vietnam, with geographical coordinates of 11°00'30" to 11°47'00" North latitude and 105°57'00" to 106°07'10" East longitude (figure 1a). The National Park has an average slope of 1 to 5 degrees, resulting in an almost flat topography with mosaics of plains, depressions and mounds forming seasonally inundated wetlands. The main soil types include typical gray soil, gray soil with red and yellow strata, alluvial soil with laterite strata and gray soil with accumulated humus on the ground. The climatic regime is ruled over by the monsoon. The wet season is from May to October, while the dry season lasts from November to April. The average temperature is approximately 27 °C, ranging from 13.0 to 39.3 °C. The annual average precipitation is 1,800 mm, with ca. 116 rainy days per year. The annual average humidity is 78.4%.

The major water sources in the National Park are the Vam Co Dong River and Da Ha – Xa Mat Stream and other small streams e.g. the Mec Nu, Sa Nghe and Thi Hang. The aquifer is shallow and slightly acidic.[7,15,23] *A. bracteosum* was mainly distributed in grasslands inside the Lo Go – Xa Mat national park[15]. The grasslands are characterized by their flat terrain, with a slope not exceeding 0.5 degrees. They serve as transitional areas for water transfer from higher regions to depressions, rivers and streams. Among the various types of

https://doi.org/10.25303/205rjbt109

wetlands in the National Park, grasslands are the most sensitive to changes in the soil's water regime. The grasslands experience short periods of flood during the wet season, while in the rest of the year, the ground is dry to arid. The vegetation in the grasslands is diverse regarding species composition including plants capable of adapting to poor nutrition and periodically dry conditions. The level of vegetation diversity depends significantly on the soil's temperature and humidity regime. Changes in the temperature and moisture can rapidly impact the plants in the areas[23].

Experimental design: The study was performed from May to December 2022, concomitant with the growing season of *A. bracteosum* in the Lo Go – Xa Mat National Park. For the species' largest cover area, San Bong (11°38'05.4"N 105°50'48.8" E) and Nhan Tran (11°37'08.9"N 105°51'51.0" E) grasslands (figure 1b) were chosen for plant growth monitoring and soil sampling to determine the plant requirements on soil conditions. Ta Not grassland (11°38'45.6"N 105°51'18.1" E) (figure 1b) was used as a control site due to the absence of the plant from this site. Five plots (0.5 m x 0.5 m) were randomly installed at each study site. From this point forward, Ta Not, San Bong and Nhan Tran grasslands are mentioned by TNO, SBO and TNR respectively.

Growth rate evaluation: The individuals' number of *A. bracteosum* was measured at each plot. Thirty individuals were selected randomly within each plot and subsequently marked to monitor the monthly changes in height during the growing season.

Soil sampling: For the abundance of species' roots in the upper 20 cm, a 2.5 cm inner diameter auger was used to collect twenty-centimeter soil cores from a plot corner with no object species' appearance. Soil temperatures and pH values from each ten-centimeter length of the soil cores were measured directly in field by a SEA/SE electrode (Schott, Germany). The soil cores were subsequently divided into two subsamples with a length of 10 cm. Wet weights of the subsamples were measured by a digital scale with two decimal places. The soil samples were stored in glass vials at 4 °C while being transported to the laboratory and conserved at -21 °C until further analysis.

Bulk density (BD) and soil water content (SWC) determination: The wet bulk density was determined according to the formula:

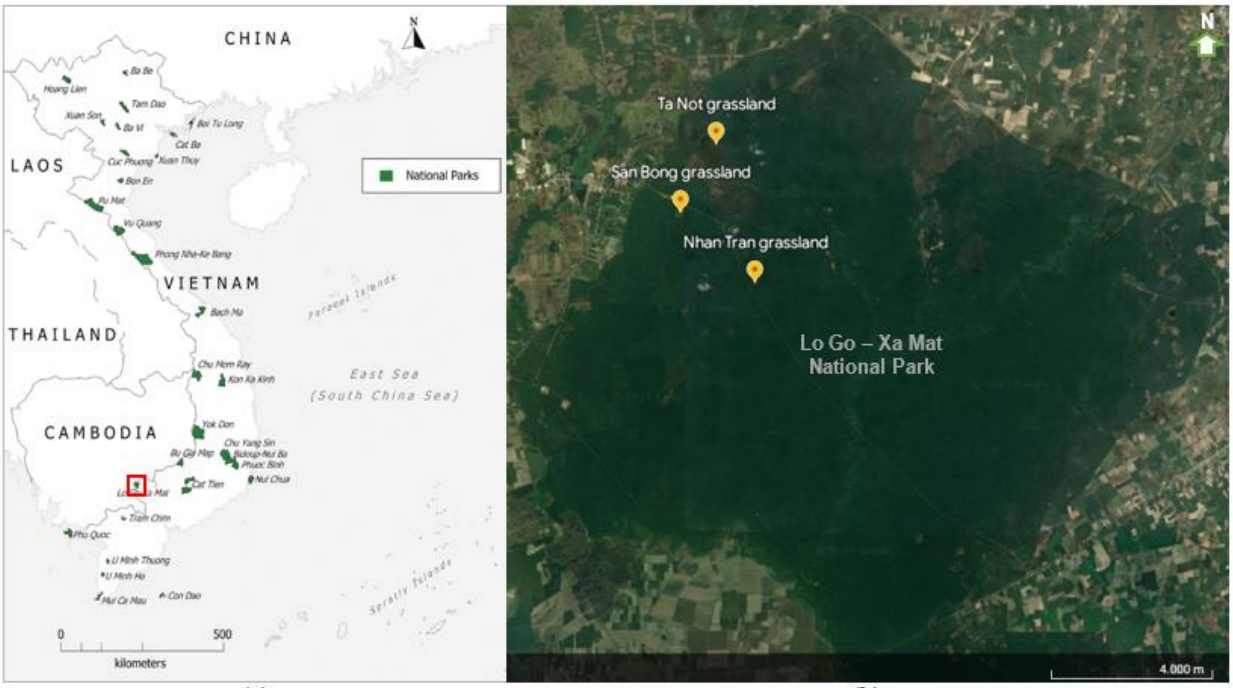
$$BD_w = \frac{m}{V}$$

where BD_w is wet bulk density (g.cm^{-3}), m is sample's wet weight (g) and V is sample volume (cm^3).

The fresh soil samples were manually well-mixed and spread on sterile plates. These plates were dried at 60 °C to constant weights. The soil water contents were calculated following the formula:

$$SWC = \frac{(P + m_1) - (P + m_2)}{(P + m_1) - P} \times 100$$

where SWC is soil water content (%), m_1 is sample's weight before drying (g), m_2 is sample's weight after drying (g) and P is plate's weight (g).



(a) (b)

Figure 1: (a) Lo Go – Xa Mat National Park in Tay Ninh Province, Vietnam[1] with (b) three study sites: Ta Not, San Bong and Nhan Tran grasslands (Resource: Google Earth)

The dried soils were homogenized by sieving through a 0.2 mm mesh size sieve to remove sand and gravel for physicochemical properties analysis. The fresh soil samples were dired at 105 ºC to constant weight. The dry bulk density was calculated in accordance with the following formula:

$$BD_d = \frac{BD_w}{1 + 0.01 \times SWC}$$

where BD_d is dry bulk density ($g.cm^{-3}$), BD_w is wet bulk density ($g.cm^{-3}$) and SWC is soil water content at 105 ºC drying (%).

Grain sizes determination: The grain sizes were determined following the process specified in the Vietnamese standard set TCVN 8567:2010. The soil grains were desorbed with hydrogen peroxide (H_2O_2), followed by carbonate removal with acetate buffer (pH = 5). The grain sizes were subsequently separated into two classes by sieving through a 0.2 mm mesh size sieve and weighed to attain the coarse and fine grain (sand and gravel, silt and clay, respectively) proportions in accordance with the following formulas:

$$\text{Coarse grain proportion (\%)} = \frac{\text{Weight of coarse grains (g)}}{\text{Weight of soil sample (g)}} \times 100$$

$$\text{Fine grain proportion (\%)} = \frac{\text{Weight of fine grains (g)}}{\text{Weight of soil sample (g)}} \times 100$$

Total nitrogen (TN) content determination: Total nitrogen contents in the dried soil samples were determined using the Kjeldahl distillation method, but the selenium (Se) catalyst was replaced by titanium dioxide (TiO_2) catalyst according to ISO 11261:1995. One gram of dry weight of each sample was digested in concentrated sulfuric acid (H_2SO_4) at 110–130 ºC for two hours. Organic substances were broken down via oxidation and reduced nitrogen was liberated as ammonium sulfate salt [$(NH_4)_2SO_4$]. The solution was subsequently distilled with sodium hydroxide (NaOH) to convert the ammonium salt to ammonia (NH_3). The distilled vapor was trapped in hydrochloric acid (HCl) and the ammonia was determined following the back titration, where part of the hydrochloric acid that was not neutralized by ammonia, was titrated with 0.1 N sodium hydroxide (NaOH).

The twice distilled water was treated with the similar process to determine the nitrogen content in the matrix. The percentage of nitrogen was calculated using the given formula:

$$TN = \frac{(V_o - V_1) \times C \times 14.0067}{m \times 1000} \times 100$$

where TN is total nitrogen content (%), V_o is volume of titrated NaOH for blank (mL), V_1 is volume of titrated NaOH for sample (mL), C is molar concentration of NaOH ($mol.L^{-1}$), 14.0067 is molar mass of nitrogen ($g.mol^{-1}$), m is dry weight of sample (g) and 1000 is transfer coefficient from mL to L.

Organic carbon (OC) content determination: Organic carbon contents of the dried soil samples were determined arccording to ISO 10694:1995. Carbonate in the samples was removed by 1 N hydrochloric acid (HCl) before being combusted at 910 °C for 6 hours to determine organic carbon. The percentage of organic carbon was calculated according to the formula:

$$OC\ (\%) = 100 \times \frac{m_1 - m_2}{m_1} \times 0.2727$$

where OC is Organic carbon (%), m_1 is Sample's weight before combustion (g), m_2 is Sample's weight after combustion (g) and 0.2727 is Transfer coefficient from CO_2 to C.

Available phosphate (AP) content determination: The PO_4^{3-} from the dried samples was extracted by Morgan solution as described by Oxmann et al[18]. Suspension was centrifuged at 3500 rpm for five minutes. The supernatant was taken and was diluted 10 times. The resulting solution was used as the analytical sample according to the description by Murphy and Riley[16].

Data analysis: Data were entered into Microsoft Excel 2016 software and statistically processed by Stagraphic Centurion XV software. The C:N ratio was determined as the ratio between carbon and nitrogen's moles. Data distribution was tested using the Shapiro–Wilk test. The One-way Analysis of Variance (ANOVA) test was used to analyze differences between places and depths. All statistical tests were performed at a significance level of 0.05.

Results

The plant growth: Figure 1 shows that the *A. bracteosum* individuals living at the NTR were significantly taller compared to the SBO during the growing season, except in August ($p < 0.05$). Additionally, the heights of *A. bracteosum* rose slowly in the first half of the growing season and increased drastically in the latter at both sites (figure 2a). The plant growth throughout the study period did not differ between these sites (figure 1a), but a higher growth rate during the early stage at the NTR was observed (figure 2b).

Despite reverse tendencies in the plant density between the study sections from June to August, the individuals' number strongly increased from August to November, especially at the SBO (figure 3). Remarkable discrepancies in the density between the sites were not found ($p > 0.05$), but the denser appearance of *A. bracteosum* at the SBO was observed over the growing season, with an exception in August (figure 3).

Soil physical properties: The soil physical properties are presented in figure 4. The soil temperature fluctuated between 33.0 and 42.0 ºC in the study sites. The maximum and minimum values were acquired at the SBO and TNO respectively. Despite the non-significant differences in the soil temperatures between depths, slightly lower temperatures were found at the beneath layer (10–20 cm) with an exception at the TNO (figure 4b). The mean soil temperature at the TNO was significantly lower than the other sites ($p < 0.05$) (figure 4b).

A significantly lower water content was recorded at the TNO compared to the others ($p < 0.05$) (figure 4a). The highest values of the soil water content (22.9 %) occurred at the SBO while the lowest (13.7 %) was found at the TNO. The water contents tended to decrease with depth at the TNO and NTR, while a contrary trend was recorded at the SBO (figure 4a), but these differences were not statistically significant ($p > 0.05$). The soils at the study area were acidic. The minimum (4.71) and maximum (6.25) values were attained at the NTR and SBO respectively. The level of acidity increased with depth at the NTR while a reverse tendency was observed at the others (figure 4c). However, no significant differences in the soil pH were found, either between depths or sites ($p > 0.05$) (figure 4c). Figure 4d shows that the soils at the TNO were probably more compact in comparison to the other sites ($p < 0.05$). The dry bulk density (BD) ranged from 1.11 to 2.11 g.cm^{-3} corresponding to the TNO and NTR. In spite of the increase in dry BD with depth at all sites (figure 4d), significant changes with depths were only acquired at the SBO ($p < 0.05$).

Grain proportions: The grain proportions are presented in figure 5. The fine grain proportions varied between 46.1% and 87.9 % recorded at the SBO and TNO respectively. According to the figure 5, the means of fine grain proportions at the TNO were significantly higher relative to the other sites ($p < 0.05$). Besides that, no remarkable discrepancies were found between the depths ($p > 0.05$) (table 1).

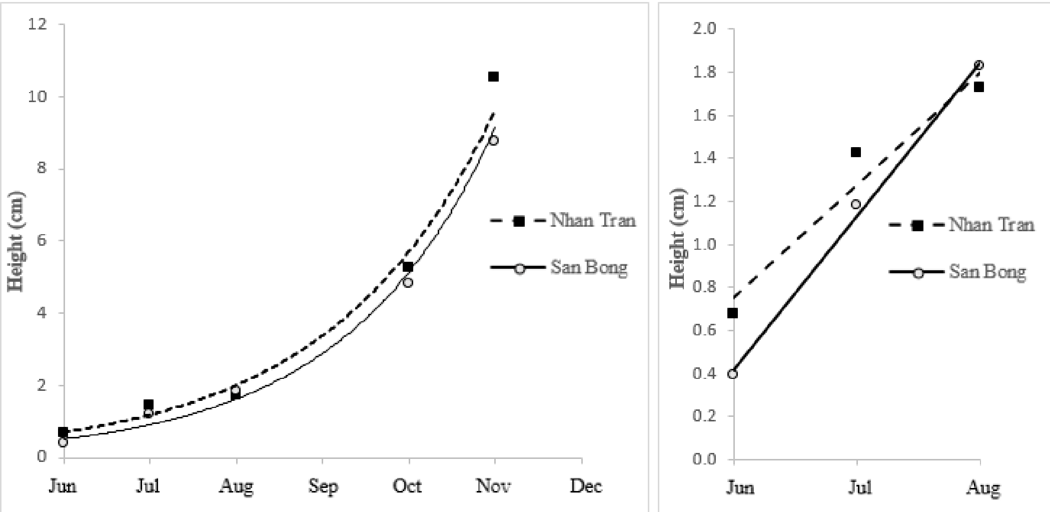


Figure 2: Temporal variation in heights of *A. bracteosum*
(a) during the growing season and (b) early growth stage at the study sites.

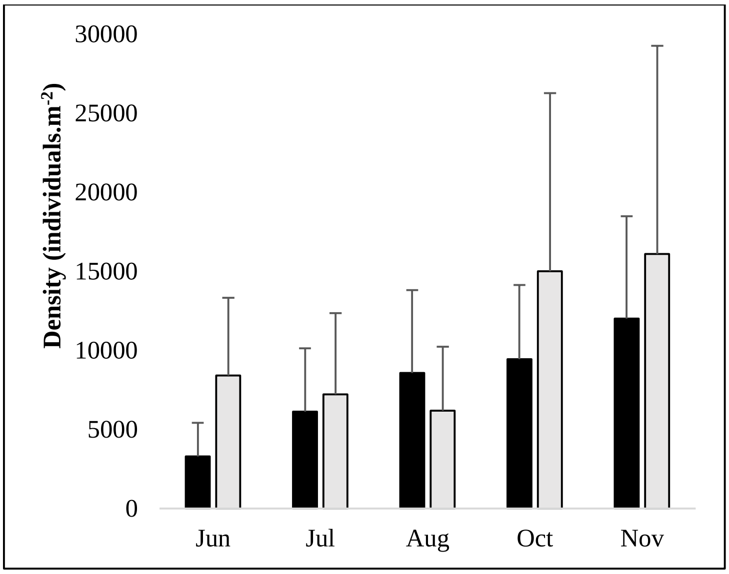


Figure 3: The temporal changes in density of *A. bracteosum* during the growing season at the study sites.
The black and gray columns represent Nhan Tran and San Bong grasslands, respectively.
The error bars represent the standard deviations of the means.

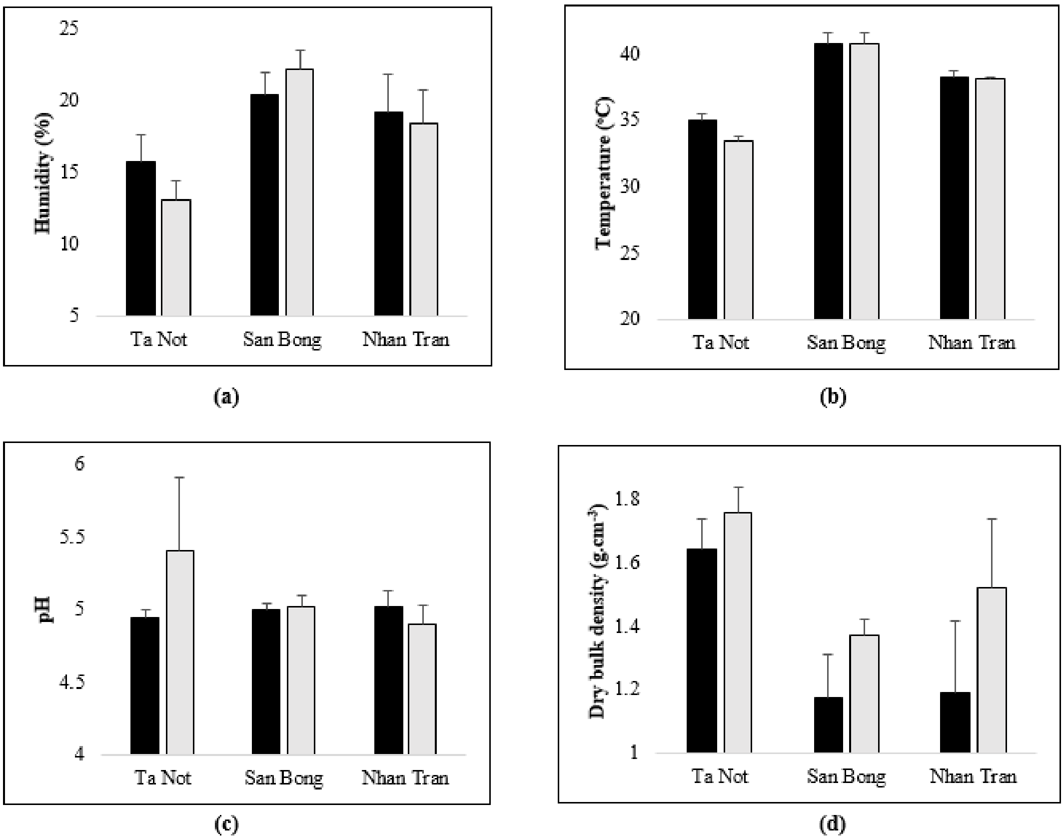


Figure 4: Depth profiles of (a) humidity, (b) temperature, (c) pH and (d) dry bulk density at the study sites. The black and gray columns represent the 0–10 and 10–20 cm layers, respectively. The error bars represent the standard deviations of the means.

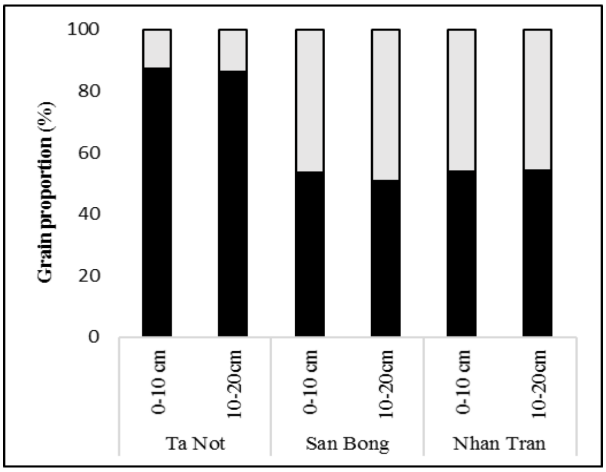


Figure 5: Vertical distribution of the fine and coarse grains in the study sites. The black and gray columns represent the fine and coarse grain proportions, respectively.

Soil elemental compositions: The soil elemental compositions are presented in figure 6. Despite a consistent vertical decrease in total nitrogen (TN) contents (figure 6a), these changes were only significant at the TNO ($p < 0.05$). The TN contents varied between 0.01 % at the TNO and 0.31 % at the SBO. The soils at the TNO seemed to be considerably poorer in nitrogen contents than the other sites ($p < 0.05$) (figure 6a). The soils at the NTR exhibited considerably poorer concentrations of organic carbon relative to the other sites ($p < 0.05$) (figure 6b). A higher accumulation of organic carbon was observed in the surface soils (0–10 cm) (figure 6b), but differences in the OC contents between the depths were only remarkable at the NTR ($p < 0.05$).

The mean of available phosphate (AP) contents at the NTR was significantly lower than at the others ($p < 0.05$) (figure 6c). The AP contents in the study area varied widely from 0.0092 $mg.g^{-1}$ at the NTR to 0.1885 $mg.g^{-1}$ at the TNO. In spite of the vertical changes in the AP levels at all sites (figure 6c), no significant differences between the depths were found ($p > 0.05$).

Soil C:N: The soils at the TNO had critically higher C:N ratios relative to the other grasslands ($p < 0.05$) (figure 7). The ratio varied drastically, from 1.24 to 124.07, as found at the NTR and TNO respectively. Despite a vertical increase tendency in the C:N value (figure 5), these differences were only considerable at the TNO ($p < 0.05$) (figure 7).

Discussion

The absence of *A. bracteosum* at the TNO probably resulted from the differences in soil physical properties between this site and the others. *A. bracteosum* was known as a plant mainly distributed in sandy-clay soil areas[2,14]. This study also showed that the plant could not grow in soils with high bulk density (BD) and fine grain proportion. In general, it was difficult for plant roots to penetrate for nutrients and water absorption from the soils with a dry BD higher than 1.6 g.cm^{-3} [19]. Thus, the extremely high dry BD values at the TNO (figure 4d) indicated that these soils were unfavorable for the plant growth. Furthermore, heavy soil texture at the TNO resulting from the high fine grain proportions (figure 5) contributed to the root growth inhibition.

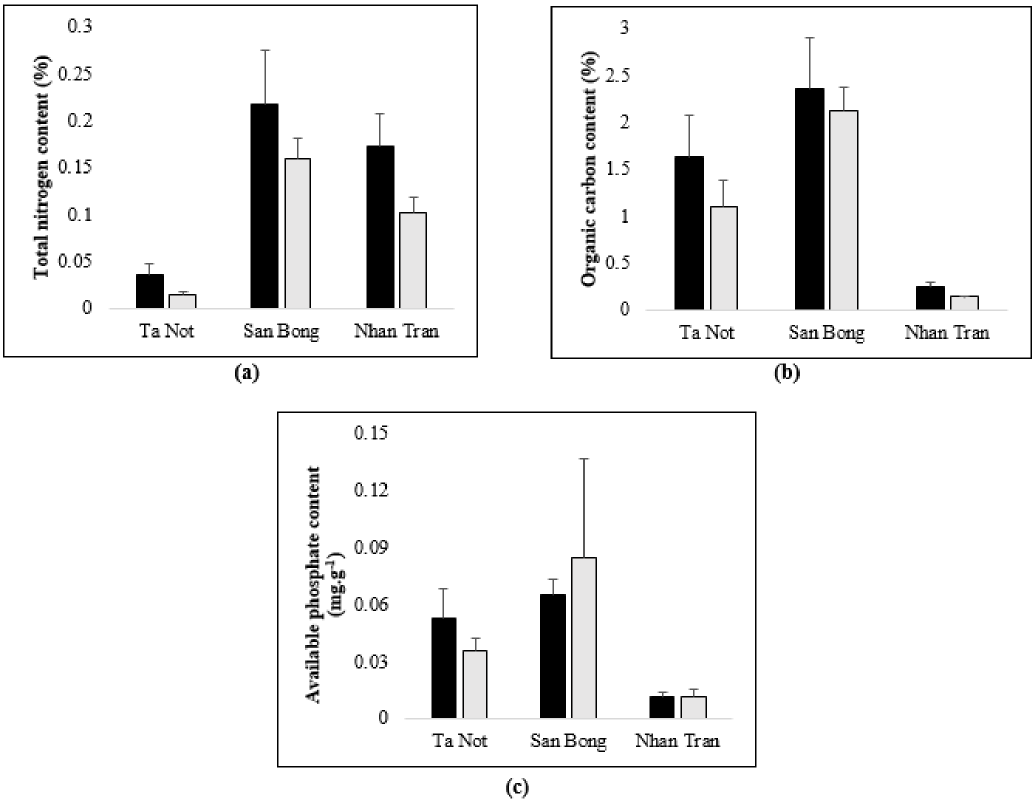


Figure 6: Vertical variation of (a) total nitrogen, (b) organic carbon and (c) available phosphate contents in the study sites. The black and gray columns represent the 0–10 and 10–20 cm layers, respectively. The error bars represent the standard deviations of the means.

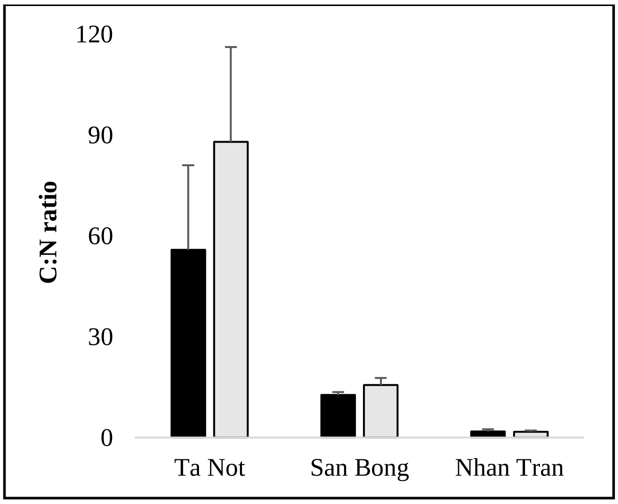


Figure 7: Discrepancies in the soil C:N ratio between depths at the study sites. The black and gray columns represent the 0–10 and 10–20 cm layers respectively. The error bars represent the standard deviations of the means.

The significantly lower soil temperatures at the TNO relative to the others (figure 4b) were caused by the thick cover of grass necromass, limiting the soil exposure to solar radiation. Furthermore, in spite of the higher dry BD and fine grain proportion (figure 5), the soils at the TNO were remarkably drier than the others (figure 4a). This condition could be caused by a canal crossing the TNO, leading to quick water loss from this site[11]. Since *A. bracteosum* is a species thriving in moist soils[2,14], the low soil humidity could be inappropriate for the plant growth and development. Additionally, the meager soil water contents might limit organic matter mineralization by microbial growth restriction (optimal soil humidity values are generally 40–60%)[26].

The study indicated that *A. bracteosum* was present at acidic soil areas (figure 4c), in agreement with previous descriptions of its habitat[2,14]. However, the non-significant differences between the study sites (figure 4c) implied that the soil pH could not be a factor that influenced the distribution of *A. bracteosum* at the study area. As phosphate is immobilized by aluminum and iron oxides in acidic soils, plants are subject to phosphorus deficiency[13,28,29]. Nevertheless, the soil pH did not directly affect the plant establishment despite the strong positive correlation between the soil pH and available phosphate (AP) concentration ($r = 0.77$, $p < 0.05$). On the other hand, the taller *A. bracteosum* individuals at the NTR (figure 2a) could be attributed to sunlight competition with other species as a consequence of high plant abundance.

The organic carbon contents at the NTR were extremely poor (figure 6b) in spite of the diverse vegetation, possibly caused by the translocation of organic matter by soil animals' activities. In addition to being a substance for phosphorus mineralization, soil organic matter is crucial in improving the AP level in soils, especially acidic soils[6,28].

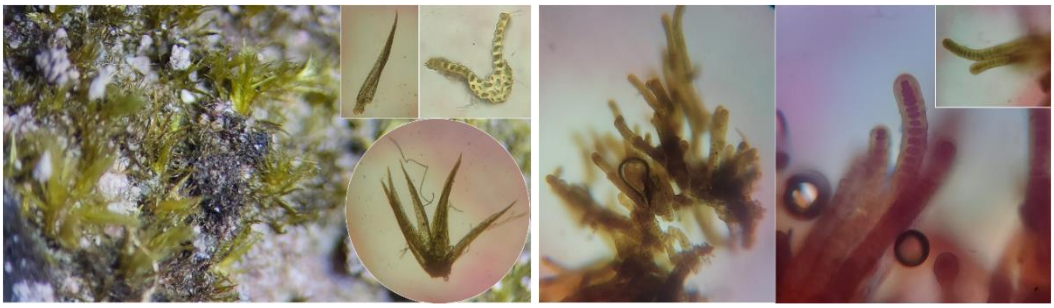
The strong positive correlation between AP and OC contents ($r = 0.94$, $p < 0.05$) in our study agreed that the competition between humic acid and phosphate for binding locations in aluminum and iron oxides leads to the higher level of AP in soils[6,28]. Consequently, the meager AP amounts at the NTR (figure 6c) might result from the extreme deficiency in organic matter inputs and the phosphate sharing between *A. bracteosum* and many other species at this site.

Both phosphorus and nitrogen are essential for plants, but nitrogen is required at a higher level[21]. The TN levels in the Lo Go – Xa Mat were lower than in Cat Tien, another National Park in southeast Vietnam[12] (table 1). However, they were comparable with other gray soils (0.030–0.121%) according to Vietnamese standards (TCVN 7373:2004), with an outlier acquired in the beneath layer at the TNO (0.010–0.017%). Therefore, the absence of *A. bracteosum* at the TNO could be attributed to the severe deficiency of nitrogen in the soils (figure 6a).

Terrestrial plants are often subject to nitrogen deficiency caused by inhibited nitrogen mineralization which is strongly influenced by the organic matter quality[5]. The organic matter decomposition rate is negatively correlated with the C:N value[3,5,17,20,22]. Microbial activities could be limited as the C:N value of the organic matter exceeds 20[17,22]. The thick grass necromass layer at the TNO probably accounted for the abundance of recalcitrant organic matter inputs, which in turn led to the drastically high soil C:N ratio (figure 7) as a consequence of the powerful microbial absorption of soil nitrogen[3,22]. Conversely, the milder conditions at the SBO and NTR resulted in the dense appearance of mosses (*Campylopus* sp.) and cyanobacteria (*Stigonema* sp.) (figure 8), a source of high-quality organic matter on the ground. Therefore, the TN contents in these soils were considerably higher than the TNO (figure 6a).

Table 1
Total nitrogen contents at 0–20 cm depth in the study areas and Cat Tien National Park

Area	Total nitrogen content (%)
San Bong	0.16–0.22
Nhan Tran	0.10–0.17
Ta Not	0.02–0.04
Cat Tien National Park	0.09–0.45



(a) (b)
Figure 8: (a) *Campylopus* sp. and (b) *Stigonema* sp. at the San Bong and Nhan Tran sites

Table 2
The appropriate soil conditions for the growth and development of *A. bracteosum* at the study sites

Soil water content (%)	18.4–22.2
Fine grain proportion (%)	50.8–54.2
pH	4.90–5.02
Dry bulk density ($g.cm^{-3}$)	1.2–1.5
Total nitrogen content (%)	0.10–0.22
Available phosphate content ($mg.g^{-1}$)	0.0113–0.0850

Surrounded by forests growing at higher elevations, the low C:N ratio at the NTR could be explained by the vertical translocation of organic matter by soil animal activities. The removal of organic matter from the study depths reduced the substance for mineralization processes and caused the low quantities of nitrogen and phosphorus at NTR compared to the SBO. The weak nutritional state, together with the abundant vegetation, led to the lower number of *A. bracteosum* individuals at the NTR relative to the SBO.

The appropriate soil conditions for the growth and development of *A. bracteosum* in the Lo Go – Xa Mat National Park are mentioned in table 2. Nevertheless, *A. bracteosum* growing at the study area was extremely short (figure 1a) and many individuals flowered at the height of ca. 2–5 cm, although the maximum height of this species was roughly 40 cm[2,4]. Consequently, the recorded conditions in Lo Go - Xa Mat national park were probably just enough to maintain the appearance of *A. bracteosum,* not optimal for the species' growth and development. The harsh conditions might relate to the drought mentioned by Huong and Son[11]. These authors claimed that the soil humidity values in the Lo Go - Xa Mat national park were substantially reduced even in the wet season. Thus, drought was probably one of the main reasons for the poor growth and development of *A. bracteosum*. Should the disadvantaged condition persist, it could lead to the disappearance of *A. bracteosum* in this national park. Hence, it is crucial to take immediate action to restore and maintain suitable environmental conditions for *A. bracteosum* in order to prevent the loss of this herb in the Lo Go – Xa Mat National Park.

Conclusion

The study showed that *A. bracteosum* in the Lo Go – Xa Mat National Park could not grow in soils with SWC < 18.4%, dry BD > 1.5 $g.cm^{-3}$, pH < 4.9, fine grain proportion > 54.2%, TN content < 0.10% and AP content < 0.0113 $mg.g^{-1}$. Notwithstanding, these conditions are not optimal for the species to thrive, for they seem to grow and develop hastily as an adaptation to the drier environment. The spatial and nutritional competition with other species at the NTR could lead to the low density of *A. bracteosum*.

Therefore, understanding the plant growth at various levels of soil conditions would be helpful to determine the optimal conditions for the plant growth and development. Furthermore, the research on the contribution of mosses and cyanobacteria to the establishment of *A. bracteosum* should enhance the probability of widening the distribution of this herb in Lo Go – Xa Mat National Park.

Acknowledgement

This research is funded by University of Science, VNU-HCM under grant number T2023-69.

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(Received 31st July 2024, accepted 07th September 2024)