

Research paper

# The Impact of Seasonal Variation of Phytoncides and Negative Air Ions on the Human Comfort Index: Implications for Planning Forest Therapy at Lienhuachih

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## ABSTRACT

This study explores environmental factors affecting phytoncides, negative air ions (NAIs) concentrations, and the human comfort index (HCI) compared and analyzed across different sampling sites and seasons to provide planning guidance for management. The research was conducted at the Lienhuachih Research Center in central Taiwan. Findings from the research revealed that the human comfort level at the Lienhuachih Research Center was relatively high in spring, summer, and fall, with the highest level observed in fall. Concentrations of phytoncides and NAIs varied with the seasons, with summer and fall recording the highest levels. The main constituents were limonene, linalool and terpinen-4-ol. The study found significant correlation between relative humidity and NAIs concentrations, with temperature being key to the thermal human comfort index though variations in the composition and concentration of phytoncides were detected at different sites. In conclusion, we recommend taking into consideration the characteristics of seasons and locations in planning optimal healing activities for forest therapy.

**Keywords:** forest therapy, phytoncides, environmental factors, negative air ions, human comfort index, Lienhuachih

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## 研究報告

# 蓮華池芬多精與負離子季節變化及人類舒適度指數 在森林療癒規劃之應用

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## 摘要

本研究旨在探討森林中的環境條件對芬多精與負離子濃度以及人體舒適度的影響，並提供相關的規劃建議。研究地點位於南投縣魚池鄉林業試驗所蓮華池研究中心之森林療癒示範基地，主要進行不同季節下林下芬多精濃度、空氣負離子濃度及人體舒適度指數等分析。研究結果顯示，芬多精之濃度於夏秋兩季較高，且以limonene、linalool與terpinen-4-ol為主要成分，應與植物的生長季節和氣象條件相關，且組成與濃度亦會隨著採樣地點不同而有所差異。環境的相對濕度對空氣中的負離子濃度有顯著影響，即空氣中負離子濃度會隨著環境相對濕度的升高而增加；而溫度則對人體舒適度有重要影響。本研究之療癒活動基地在春、夏、秋季節對人體舒適度均極高，感受上屬於「非常舒服」的等級，其中尤以秋季為最佳。因此，規劃蓮華池森林療癒基地之活動而言，以夏秋兩季為佳，建議在活動規劃時應考慮季節及樣點的特性，以提供最佳的療癒效果。

關鍵詞：森林療癒、芬多精、空氣負離子、環境因子、人體舒適度、蓮華池

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## INTRODUCTION

In recent years, forest therapy activities have received much attention in academia for their rejuvenation and promotion of overall physical, mental, and emotional well-being through connection with nature. Studies have shown that engaging in forest-based activities can activate the subgenual prefrontal cortex, suggesting a positive impact on spiritual regulation (Bratman et al. 2015), and enhance the activity and number of natural killer cells in the body, an effect lasting for 7 and up to 30 days (Li 2010, Park et al. 2010, Yu et al. 2017). Taking into account environmental background

information to inform activity and trail traffic flow design is essential to optimize the health benefits for participants and maximize therapeutic forest immersion. As observed in the United States Forest Service's practices, a crucial aspect of developing forest therapy involves identifying the features of forest environments and using this information to create design, planning, and management recommendations (Gobster et al. 2023). The Lienhuachih Research Center of the Forestry Research Institute has been a designated a demonstration site for forest therapy since 2016 and offers a variety of healing

programs. These initiatives have included designing healing courses, training of forest therapy guides, and curating healing meals. Physiological and psychological assessments conducted before and after participation in these programs support the effectiveness of forest therapy (Lin et al. 2018). However, existing research focuses primarily on the significance of the effects of forest therapy activities, while comprehensive data related to site-specific environmental factors and human comfort levels remain lacking. For this reason studying environmental variation at therapy sites is essential to providing comprehensive information for activity planners and participants.

Derived from plants, phytoncides are volatile compounds whose constituents, amounts and effects vary with plant species, seasons, and forest structures. These compounds have been recognized as important components benefiting forest activities for almost a century (Alves et al. 2016, Chen et al. 2019, Simpraga et al. 2019, Zhu et al. 2021, Li et al. 2022). While extensive research has been conducted on the physical and mental benefits of forest therapy activities (Zhu et al. 2022, Chen et al. 2023, Subirana-Malaret et al. 2023), little attention has been paid to on-site planning based on environmental characteristics. Zhu et al. (2021) monitored and compared factors such as negative air ions (NAIs) concentration, oxygen levels, the human comfort index (HCI), and relative phytoncide content in various forest types within Shimen National Forest Park, offering recommendations for optimal forest tourism seasons and activity planning. However, domestic research on the concentration of phytoncides and their relationship to environmental factors in forests remains quite limited. Future studies focusing on measurement of phytoncides on-

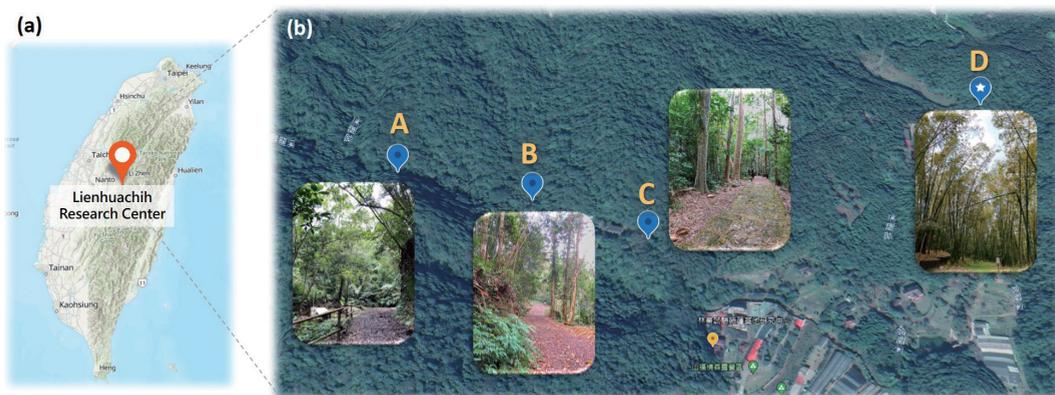
site in forests are imperative to improving this understanding.

This study focuses on monitoring phytoncide and NAIs concentration at the Lienhuachih Research Center in Nantou County, Taiwan, to provide environmental information from the forest site for forest therapy planning. Seasonal monitoring was conducted to analyze variations in concentrations across sampling sites. Research aims were to establish a scientific basis for forest therapy activity planning by exploring how phytoncides are affected seasonally by NAIs, and environmental factors at different sites. This study is the first to quantitatively measure phytoncide concentrations and analyze their compositions along domestic forest trails. Unlike data on the relative content of phytoncides, the results obtained in this study provide valuable information about phytoncide composition and concentrations at different locations and times to be used for planning improvement and future reference and comparison.

## MATERIALS AND METHODS

### Phytoncides concentration measurement in different seasons and on different forest trails

To understand the phytoncide composition and seasonal variation on different forest trails at Lienhuachih Research Center, this experiment was carried out at four sampling sites (Fig. 1): Site A (located at the No. 2 water weir of the Lianhuachi Research Center), Site B (a forest trail featuring the unique Taiwanese species *Mitrastemon kanehirai*), Site C (a forest trail leading to the log cabin classroom), and Site D (characterized by a forest of Giant Bamboo *Dendrocalamus giganteus*). Beginning in 2018, dynamic monitoring of phytoncide concentrations was carried out at each sampling site during



**Fig. 1.** Map of the study area. (a) The study site is located at the Lienhuachih Research Center in central Taiwan. (b) Locations of phytoncide and NAIs sampling sites.

each of the four seasons: winter (January), spring (April), summer (July) and fall (October). Employing Tenax TA adsorbents (60–80 mesh, Supelco, Bellefonte, PA, USA), a custom-designed automated timed sampling system was used to collect air from each site at a flow rate of  $150 \text{ mL min}^{-1}$ , accumulating 36 L of air for each sample. At each site, six samples were collected daily (one sample every four hours). Seven to fourteen consecutive sampling days were conducted per season, spanning three years. Values from different seasons and trails were presented as the mean of the samples. The adsorbent collection tubes were stored at  $4^\circ\text{C}$  and qualitative and quantitative analyses were completed within one week.

### Gas chromatography-mass spectrometry analysis

Following sample collection, samples obtained were preprocessed using an automatic thermal desorption instrument (ATD) coupled with an automated internal standard gas addition system. Then, gas chromatography-mass spectrometry (GC-MS) analysis was conducted using a Clarus

600 system (PerkinElmer). The separation column was a DB-5ms column (crossbond 5% phenyl methylpolysiloxane) 30 m in length, with a 0.25 mm inner diameter, and 0.25  $\mu\text{m}$  film thickness. GC-MS parameters were as follows: injection temperature of  $250^\circ\text{C}$ , carrier gas: helium at a flow rate of  $1 \text{ mL min}^{-1}$ , ion source temperature of  $230^\circ\text{C}$ , and a split ratio of 20:1. The GC oven temperature program started at  $50^\circ\text{C}$ , held for 1 minute, followed by a temperature ramp of  $4^\circ\text{C min}^{-1}$  to  $200^\circ\text{C}$ , and a ramp in the 2<sup>nd</sup> phase to  $250^\circ\text{C}$  at  $20^\circ\text{C min}^{-1}$ , then held for 3 minutes. Compound identification was made by comparing mass spectra and algorithmic index (AI) with a mass spectra library and reference AI (rAI) (Adams 2007). MS databases used included the Wiley/NBS Registry of Mass Spectral Data (version 7) and NIST MS Search (version 2). The spectra of external standards for commercially available compounds were also employed to identify and quantify the chemical composition of phytoncides, including  $\alpha$ -pinene (98%, Acros), camphene (96%, ICN), sabinene (92%, SG),  $\beta$ -pinene (98%, Acros),  $\beta$ -myrcene (90%, Sigma),  $\delta$ -3-carene (90%, Acros),

limonene (92%, Acros),  $\alpha$ -phellandrene (90%, TCI), *trans*- $\beta$ -ocimene (90%, SAFC),  $\gamma$ -terpinene (98%, Acros), terpinolene (95%, TCI), linalool (95%, Acros), terpinen-4-ol (97%, Acros),  $\alpha$ -terpineol (99%, Acros), and  $\alpha$ -cedrene (99%, Aldrich). Standards in methanolic solution were injected into adsorbent cartridges in a helium stream. The absolute quantification of the phytoncides was performed by establishing a calibration curve using standard samples of different concentrations. The peak area of the phytoncides was then substituted into the calibration curve to calculate the concentration of the terpene component.

#### Monitoring of NAIs and meteorological data

During the sampling period, NAIs concentrations were monitored at fixed locations 1 meter above ground, with data readings taken in the four cardinal directions (north, south, east and west). Each measurement was repeated three times after zeroing, and the process was carried out once in the morning and again in the afternoon each day. Concentration of NAIs was measured with a standardized and calibrated COM-3800 V2 negative ion monitor (Com System Inc., Japan), with an observation range of 0 to  $5.0 \times 10^6$  ions  $\text{cm}^{-3}$  and measurement accuracy of  $\pm 5\%$ . Environmental parameters, including ambient temperature ( $^{\circ}\text{C}$ ), relative humidity (RH%), and photosynthetic active radiation (light intensity) (PAR,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), were recorded using a WatchDog 1000 Series data logger (Spectrum Technologies, Aurora, IL, USA) at 10-minute intervals. Data was collected continuously for 7 to 14 consecutive days each season.

#### Calculation of HCI

Calculation of HCI is based on the research of Lu et al. (1984), who integrated

data from theories of environmental hygiene to comprehensively evaluate the effect of environmental factors including temperature, relative humidity, and wind speed on human comfort. The HCI formula, which has been widely adopted, is as follows (Lu et al. 1984):

$$\text{HCI} = 0.6 \times |T - 24| + 0.07 \times |\text{RH} - 70| + 0.5 \times |V - 2|$$

Where HCI represents the human comfort index, T denotes the environmental temperature in degrees Celsius ( $^{\circ}\text{C}$ ), RH is relative humidity expressed as a percentage (%), and V represents average wind speed in meters per second ( $\text{m s}^{-1}$ ). A lower HCI value corresponds to a higher level of comfort. Definitions for different ranges of HCI are as follows:

$\text{HCI} \leq 4.55$ : Very comfortable

$4.55 < \text{HCI} \leq 6.95$ : Comfortable

$6.95 < \text{HCI} \leq 9.00$ : Uncomfortable

$\text{HCI} > 9.00$ : Extremely uncomfortable.

#### Data processing and analysis

The comparison of various parameters in this study (changes in average temperature, relative humidity, and concentration of NAIs across different seasons and sites) was performed using IBM SPSS Statistics software, version 29.0. Comparison was performed through one-way analysis of variance (ANOVA) using Scheffé's post hoc test to examine whether significant differences exist. Multiple regression analysis was employed, with NAIs concentration as the dependent variable and environmental factors including temperature, light intensity, and relative humidity as independent variables. These factors were correlated with NAIs concentrations, to identify significant factors.

## RESULTS

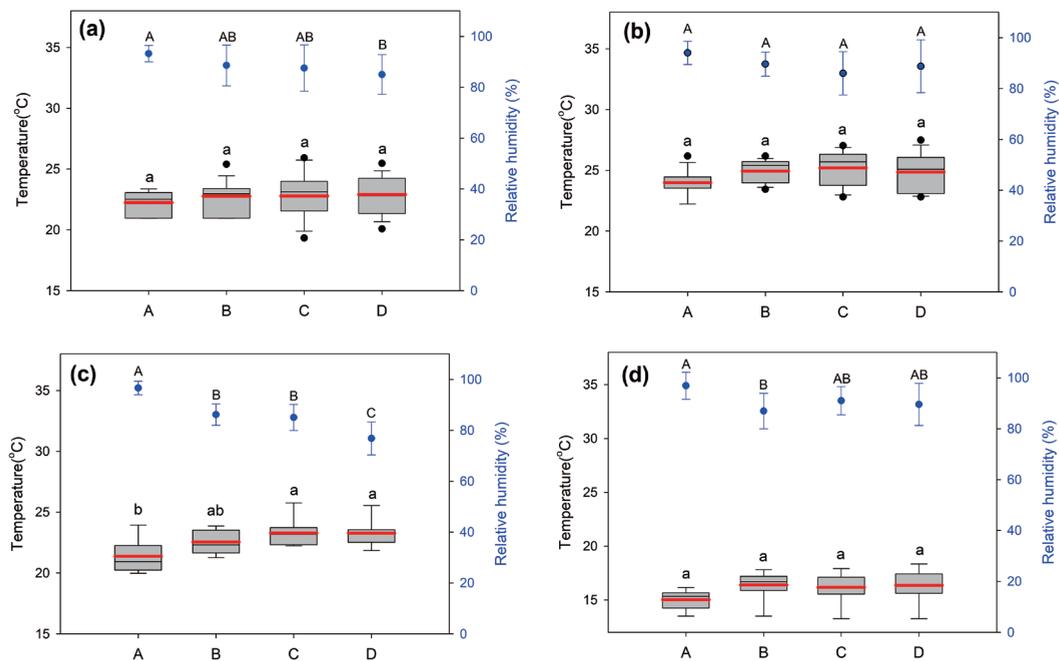
### Differences in HCI across different seasons and sites

The Lienhuachih Research Center is located in the low-altitude mountainous area of Nantou County, with elevations ranging from 576 to 925 meters. The monitoring of this study found that the average temperatures at the 4 sampling sites during spring and fall were approximately 20 - 24°C (Fig. 2). The average temperature rose slightly to around 24 - 26°C during summer, while there was a noticeable decrease in temperature during winter, with an average temperature of approximately 14 - 18°C. Among the 4 sampling sites, site A generally had slightly lower temperatures than the other sites, except in fall; however, statistically significant temperature differences between the sampling

sites were not observed, except during the fall. Generally, the environmental temperature comfortable to humans is 22°C to 26°C (ASHRAE 2017, Cui et al. 2013), therefore the Lienhuachih Research Center enjoys a cool and pleasant climate environment almost year round.

Relative humidity at the Lienhuachih Research Center exceeds 80% throughout the year. Site A consistently recorded higher relative humidity than the other sites, exhibiting significant differences during spring, fall, and winter (Fig. 2).

Light intensity was below 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 3) at each sampling site due to tree canopy attenuation. Of all the sampling sites, site D ( $77.1 \pm 33.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) showed slightly higher light intensity than the other sites ( $46.2 \pm 16.7 - 57.7 \pm 27.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ); however, no statistically significant



**Fig. 2.** Seasonal variation of average temperature and light intensity at different sampling sites. (a) Spring, (b) Summer, (c) Fall, and (d) Winter.

differences in average light intensity were observed between the sampling sites (Fig. 3a). Across different seasons, average light intensity was significantly higher during summer ( $84.9 \pm 25.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to the other seasons ( $32.0 \pm 11.2 - 66.7 \pm 17.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Fig. 3b).

The combined effect of these environmental factors is critical to determining perception of human comfort. HCI was calculated in the different seasons and at the different sampling sites using the HCI formula (Lu et al. 1984, Zhu et al. 2021), integrating temperature, relative humidity, and wind speed. The results (Fig. 4) reveal

that during spring ( $\text{HCI} = 2.5 \pm 0.7 - 3.3 \pm 0.0$ ), summer ( $\text{HCI} = 2.3 \pm 0.4 - 2.5 \pm 0.1$ ), and fall ( $\text{HCI} = 1.5 \pm 0.8 - 3.9 \pm 1.2$ ), HCI values for all sampling sites remained below 4.55, corresponding to an “extremely comfortable” environment. However, during winter, the comfort index ranged from  $6.4 \pm 0.7$  to  $7.6 \pm 0.7$ , falling within the range of “comfortable” to “uncomfortable” on the HCI scale.

**Seasonal variation of NAIs concentration at different sites**

The average concentrations of NAIs across the four sampling sites and seasons are

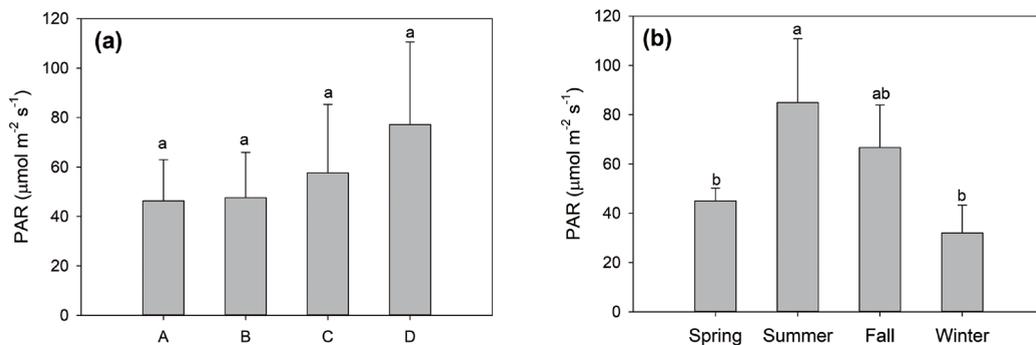


Fig. 3. Average light intensity at different sampling sites (a), in different seasons (b)

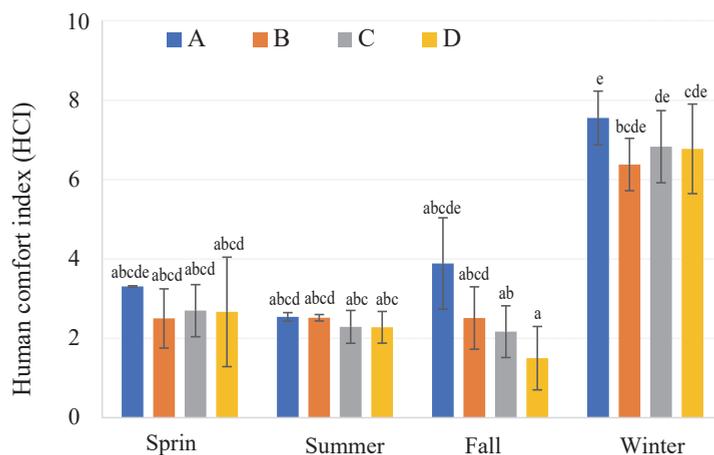


Fig. 4. Human comfort index (HCI) of each sampling site in different seasons.

shown in Fig. 5a. NAIs concentrations during summer were significantly higher than during spring, fall and winter ( $p < 0.01$ ). NAIs concentrations in different seasons ordered by concentration were: summer  $1088 \pm 236$  ions  $\text{cm}^{-3}$  > fall  $718 \pm 90$  ions  $\text{cm}^{-3}$  > winter  $668 \pm 25$  ions  $\text{cm}^{-3}$  > spring  $629 \pm 61$  ions  $\text{cm}^{-3}$ . Nevertheless, there were no significant differences in the average concentration of NAIs between different sites. Concentration

levels ordered from highest to lowest were: site A  $1014 \pm 253$  ions  $\text{cm}^{-3}$  > site B  $765 \pm 103$  ions  $\text{cm}^{-3}$  > site C  $729 \pm 57$  ions  $\text{cm}^{-3}$  > site D  $595 \pm 67$  ions  $\text{cm}^{-3}$  (Fig. 5b).

### Seasonal phytoncide composition and concentration variation at different sites

The phytoncide composition across different seasons and sampling sites are illustrated in Fig. 6. During spring, the

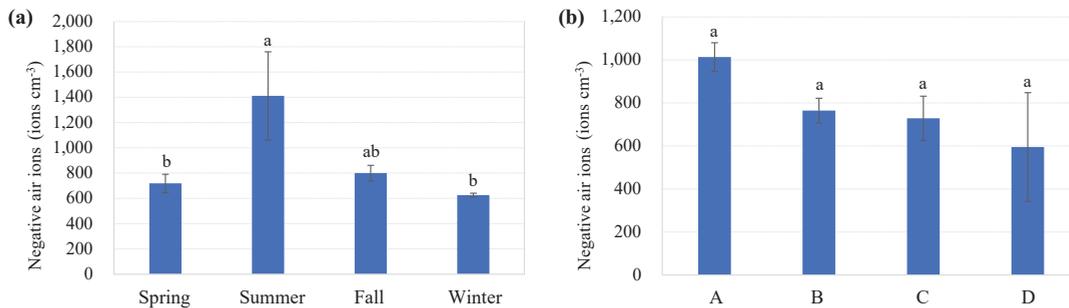


Fig. 5. Average NAIs concentration in different seasons (a) and at different sampling sites (b).

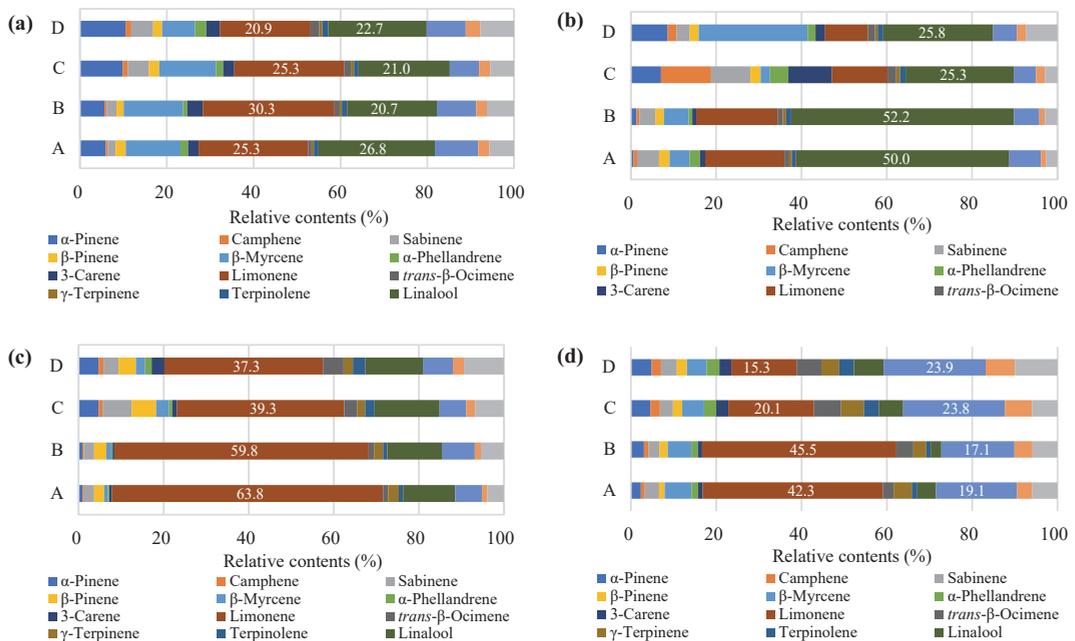


Fig. 6. Seasonal variation and chemical composition of phytoncides at different sampling sites. (a) Spring, (b) Summer, (c) Fall, and (d) Winter.

phytoncide composition at the four sampled sites was dominated by limonene ( $20.9 \pm 6.9\% - 30.3 \pm 13.8\%$ ) and linalool ( $20.7 \pm 10.4\% - 26.8 \pm 12.9\%$ ). In summer, there was a noticeable increase in the proportion of linalool ( $25.3 \pm 11.9\% - 52.2 \pm 25.1\%$ ). During fall, phytoncide compositions were predominantly rich in limonene ( $37.3 \pm 0.1\% - 63.8 \pm 9.4\%$ ) at all sampling sites. In winter, limonene ( $15.3 \pm 9.8\% - 45.5 \pm 34.1\%$ ) remained the main component of phytoncides, while there was an increase in the relative content of terpinen-4-ol ( $17.1 \pm 12.1\% - 23.9 \pm 0.3\%$ ). Furthermore, as is seen from Fig. 6, phytoncide compositions were quite similar between sites C and D, as well as between sites A and B.

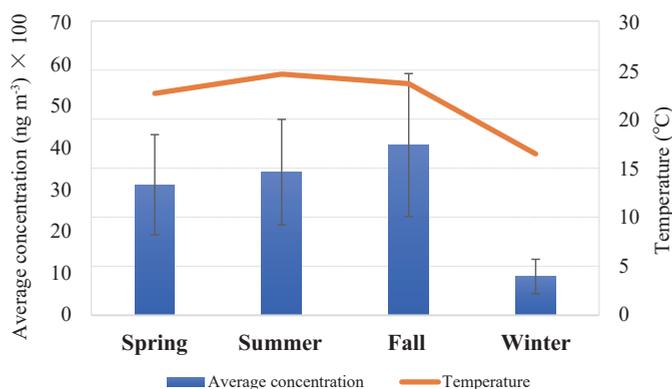
Average phytoncide concentrations across different seasons at Lienhuachih Research Center are shown in Fig. 7. The concentrations of phytoncides across the four seasons exhibit a trend similar to seasonally fluctuating temperatures, ranked as follows: fall ( $4053.7 \pm 1700.6 \text{ ng m}^{-3}$ ) > spring ( $3105.9 \pm 1193.7 \text{ ng m}^{-3}$ ) > summer ( $3406.3 \pm 1256.1 \text{ ng m}^{-3}$ ) > winter ( $923.0 \pm 411.4 \text{ ng m}^{-3}$ ).

The average concentration of phytoncide during winter were significantly lower than in the other three seasons. Seasonal variation in phytoncide concentrations at the four sampling sites are shown in Fig. 8. Regardless of the season, phytoncide concentrations were consistently higher at site A ( $1153.2 \pm 327.5 - 8179.6 \pm 5138.0 \text{ ng m}^{-3}$ ) and site B ( $1982.1 \pm 1618.3 - 6193.2 \pm 4203.3 \text{ ng m}^{-3}$ ) compared to site C ( $390.0 \pm 155.8 - 1641.4 \pm 835.0 \text{ ng m}^{-3}$ ) and site D ( $166.6 \pm 94.4 - 1306.5 \pm 1149.4 \text{ ng m}^{-3}$ ). The concentrations of phytoncides varied among different sampling sites, ranging from 15 to 49 times, indicating notable differences not only in phytoncide composition but also in concentration.

## DISCUSSION

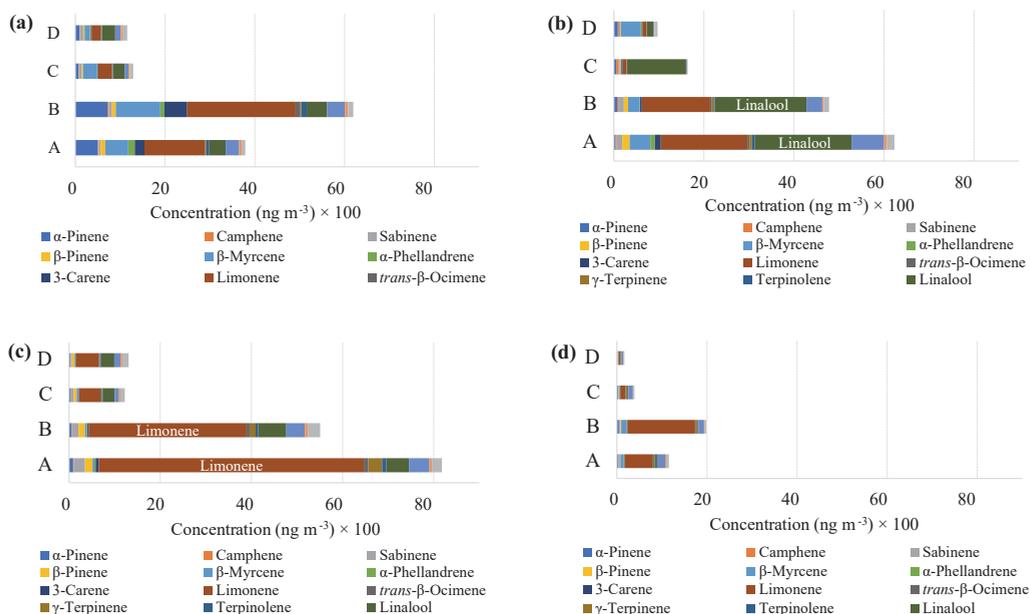
### Differences in human comfort index across different seasons and sites

Overall, the HCI at the four research sites of Lienhuachih Research Center generally falls within the “comfortable” or higher range. Except for a slightly lower HCI during winter, each site provides a comfortable



**Fig. 7.** Changes in the average concentration of phytoncides at Lienhuachih Research Center over four seasons.

\* Values represent averages from all sampling sites recorded during the same season over the three-year period.



**Fig. 8.** Seasonals variation of average phytoncide concentration at different sampling sites: (a) Spring, (b) Summer, (c) Fall, and (d) Winter.

experience in spring, summer, and fall (Fig. 4). Many research studies indicate that various environmental factors affect the HCI. Among these factors, temperature is widely acknowledged as crucial in determining human comfort (Ruiz and Correa 2015, Teshnehdel et al. 2020, Zhu et al. 2021). In multivariate regression analysis involving environmental factors including temperature (T), relative humidity (RH), and wind speed (V) in relation to HCI, only temperature and relative humidity significantly contribute to HCI ( $p < 0.01$ ), accounting for 95.7% of the variation in HCI. Wind speed, on the other hand, is considered an insignificant variable ( $p > 0.05$ ), and the optimal regression model is  $-0.541 \times T + 0.068 \times RH + 9.402$  (Table 1). Standardized coefficients reveal that temperature has the most significant impact on changes in HCI, followed by relative humidity.

This study found that average tempera-

tures ranging from 20 to 25°C during spring, summer, and fall across all sampling sites lead to consistently “very comfortable” conditions with HCI values below 4.55. Notably, Site D showcases exceptional comfort potential in fall (HCI =  $1.5 \pm 0.8$ ), presenting the lowest relative humidity (RH =  $76.8 \pm 6.5\%$ ) and a highly agreeable temperature ( $23.3 \pm 1.1^\circ\text{C}$ ), fostering an optimal comfort sensation for humans. This optimal comfort is supported by the ASHRAE guidelines, which recommend a comfortable temperature range of 23 - 26°C for summer and 20 - 23.5°C for winter, with humidity between 30% and 65% (ASHRAE 2017). Conversely, winter experiences a significant reduction in HCI due to the drop in average temperatures.

Site A exhibited lower HCI values than the other three sites in all seasons. The underlying reason is high relative humidity (above 93%) throughout the year, higher than the other sites (76.8% - 91.0%), especially

**Table 1. Multiple regression relationships between meteorological variables and human comfort index (HCI)**

Model	Unstandardized coefficients		Standardization coefficients Beta	T	Significance
	B	Standard error	$\beta$		
(Constant)	9.402	1.544		6.089	0.000
Wind speed	-0.246	1.390	-0.011	-0.177	0.861
Relative humidity	0.068	0.016	0.180	4.190	0.000
Temperature	-0.541	0.038	-0.917	-14.059	0.000

Dependent variable: human comfort index.

during winter, where the combination of high humidity and low temperatures results in uncomfortable HCI indices.

#### Seasonal variation of NAIs concentration and its correlation with environmental factors

The findings of this study reveal pronounced seasonal variations in the concentration of NAIs at different sampling sites within the Lienhuachih Research Center. Several studies have indicated that seasonal fluctuations in NAIs concentrations may be attributed to changes in environmental factors such as vegetation growth, radiation conditions, temperature and humidity (Jiang et al. 2018, Zhu et al. 2021). The results obtained in this study reveal that the average concentration of NAIs at each sampling site is highest during the summer, followed by fall, and lowest in spring and winter. This trend is consistent with numerous previous studies (Pan et al. 2012, Li et al. 2022). Li et al. (2022) which conducted long-term monitoring of the seasonal variation of NAIs in urban green spaces in subtropical regions of China found that the average concentrations of NAIs in spring, summer, fall and winter were in the order of  $680 \text{ ions}\cdot\text{cm}^{-3}$ ,  $735 \text{ ions}\cdot\text{cm}^{-3}$ ,  $703 \text{ ions}\cdot\text{cm}^{-3}$ , and  $685 \text{ ions}\cdot\text{cm}^{-3}$ , respectively, with significantly higher

concentrations observed during summer.  $\text{O}_2^{\cdot-}$  is one of the most common NAIs, primarily generated in the thylakoid membranes of photosystem I (PSI) (Scarpeci et al. 2008) or photosystem II (PSII) (Cleland and Grace 1999) in plants. Plant peroxidases binding to cell wall polymers through ionic or covalent bonds located in the apoplast, may contribute to generating extracellular superoxide ions (Bolwell et al. 1998, Jiang et al. 2018). Many studies suggest that higher concentrations of NAIs in summer may be attributed to more intense ultraviolet radiation and enhanced photosynthetic activity of plants, leading to more substantial photoelectric effects, facilitating the release of negative ions from leaf tips (Li et al. 2019, Shi et al. 2021, Li et al. 2022).

Regarding the variation in NAIs concentrations between sampling sites, although statistical significance was not observed in this study, the average NAIs concentration at sampling site A was higher than at the other three locations. Previous related research has confirmed that dynamic flowing water bodies such as rivers, waterfalls, and water sources induce ionization effects due to charge separation upon water droplet fragmentation (known as the Lenard effect), leading to a significant

increase in NAIs concentrations (Kolarž et al. 2012, Zhang et al. 2016). As site A is near a weir and has abundant water resources compared to the other three locations, this could explain the elevated NAIs concentrations at this site.

Additionally, various environmental factors including T, RH, and PAR, were incorporated into multiple regression analysis to gauge their effect on NAIs concentrations (Table 2). The results showed that only RH significantly contributed to variation in NAIs concentrations ( $p < 0.05$ ), explaining 40.7% of the variability, while the other two factors were considered minor influences ( $p > 0.05$ ). The optimal regression model was  $\text{NAIs} = 0.588 \times \text{RH} - 3311.179$  (Table 2). This shows that relative humidity and NAIs concentration are significantly positively correlated, and the higher the RH, the higher the NAIs concentration. This is consistent with most other research findings (Zhang et al. 2011, Li et al. 2022). The relationship between NAIs and environmental humidity has been a focal point of many studies (Wang et al. 2004, Miao et al. 2018, Yu et al. 2018, Luo et al. 2020). Generally, NAIs are composed of multiple negatively charged molecules, which form negative ion clusters by associating with numerous water molecules, typically

around 20 to 30, such as  $\text{CO}_3^-(\text{H}_2\text{O})_n$ ,  $\text{O}^-(\text{H}_2\text{O})_n$ ,  $\text{O}_3^-(\text{H}_2\text{O})_n$ ,  $\text{O}_2^-(\text{H}_2\text{O})_n$ ,  $(\text{OH})^-(\text{H}_2\text{O})_n$ , and  $\text{CO}_4^-(\text{H}_2\text{O})_n$ , etc. (Jiang et al. 2018, Luo et al. 2020). When environmental humidity increases and atmospheric water vapor content rises, free electrons released through the ionization of neutral molecules tend to attach to water molecules, leading to the formation of NAIs (Smirnov 1992, Wang et al. 2020). Moreover, elevated humidity reduces transpiration from plant leaves while promoting stomatal opening and enhancing photosynthesis, resulting in increased production of NAIs (Miao et al. 2018). With increasing relative humidity, water vapor in the air condenses into droplets, contributing to atmospheric particle purification and prolonging the existence of NAIs (Luo et al. 2020).

#### Variation in phytoncide composition and concentration across seasons at different sites

Phytoncides are volatile compounds primarily composed of terpenoids that plants produce as a defense mechanism against pathogenic microbial invasions or as signaling compounds. Terpenoids are composed of isoprene units with five carbons each, and can be regarded as a class of compounds formed by the connection of

**Table 2. Multiple regression relationships between light intensity, temperature, relative humidity, and NAIs**

Model	Unstandardized coefficients		Standardization coefficients Beta	T	Significance
	B	Standard error	$\beta$		
(Constant)	-3311.179	1452.478		-2.280	0.042
PAR	-0.931	3.800	-0.080	-0.245	0.811
Temperature	45.590	28.241	0.523	1.614	0.132
Relative humidity	35.550	14.294	0.588	2.487	0.029

Dependent variable: negative air ions (NAIs).

\* PAR: light intensity (photosynthetic active radiation).

two or more isoprene units in various ways. Depending on the number of isoprene units, terpenoids are classified into monoterpene ( $C_{10}$ ), sesquiterpene ( $C_{15}$ ), diterpene ( $C_{20}$ ), triterpene ( $C_{30}$ ), and tetraterpene ( $C_{40}$ ), among others. Among these, the more volatile terpenoids consist mainly of monoterpene and sesquiterpene compounds (Maffei et al. 2011). Many terpenoids have antibacterial or antimicrobial functions (Simpraga et al. 2019), and some research results have also demonstrated their potential to induce calming, comforting, and relaxing effects in humans (Dayawansa et al. 2003, Simpraga et al. 2019, Lau et al. 2021).

At the four sampling sites of the Lienhuachih Research Center, the composition of phytoncides is mainly dominated by monoterpene compounds such as limonene and linalool. The composition of phytoncides varies between sampling sites, with sites C and D sharing similar compositions, as do sites A and B. In terms of vegetation ecology, sample sites A and B are both dominated by broad-leaved trees, with similar vegetation. Site C is characterized by *Eucalyptus citriodora*, and site D is a single forest of giant bamboo. In addition to their close geographical proximity, sites A and B also have similar vegetation ecology; therefore the composition of their phytoncides is also more similar. In general, biogenic volatile organic compounds (BVOCs) vary considerably across plant species and vegetation types (Kesselmeier and Staudt 1999). This study also found similar results. Variation in phytoncide concentrations at the four sampling sites across different seasons showed that differences in vegetation composition not only affect the composition of phytoncide species, but also has a significant impact on overall release concentrations. This is likely due to the fact that different enzymes respond differently to temperature,

resulting in different phytoncide components being emitted from plants under different temperature conditions (Xu et al. 2010, Chen et al. 2022). Zhu et al. (2021) compared the phytoncide composition and concentration of five different forest types (*Phyllostachys edulis* forests, subtropical evergreen broad-leaved forests, *Liquidambar formosana* forests, *Cunninghamia lanceolata* forests, and coniferous and broad-leaved mixed forests). They found that the subtropical evergreen broad-leaved forest had significantly more species and a higher relative content of phytoncides than other forest types. This may be attributed to the mature and natural secondary forest community, which features rich vegetation types, a complete community structure, high forest coverage, and canopy density. These factors likely contribute to the production of more diverse and abundant phytoncides (Zhu et al. 2021). This may also explain why the total concentration of phytoncides in a bamboo forest with a single vegetation type is far lower than that of the other three sampling sites.

Generally, the composition of volatile compounds released by plants reflects not only the physiological needs of the trees, but also their ecological responses within the ecosystem. The roles of identical components can differ between different tree species (Rivoal et al. 2010, Matsunaga et al. 2011, Mochizuki et al. 2011, Helmig et al. 2013). For example, limonene is a significant component often released by the leaves of many plants and is ubiquitous in the air, while linalool is a primary component released by the flowers of many flowering plants. Therefore, the composition of phytoncides in the Lienhuachih Research Center may be correlated with the vegetation composition at each sampling site and with the seasonal phenological changes of these plants. The

increase in linalool content in summer could be related to the phenological changes of the plant community in the experimental area, although more comparisons are required to confirm.

Seasonal variation of phytoncide concentration is positively correlated with seasonal temperature changes. This result is consistent with research by Chen et al. (2019), which demonstrated that higher temperatures led to an increased emission rate of phytoncides from plants. Moreover, similar results have been observed in other studies, such as those on *Pinus longaeva*, *Picea pungens*, *P. halepensis*, *Pseudotsuga menziesii*, and *Quercus suber*, which all reach highest emission rates during the summer (Pio et al. 2005, Helmig et al. 2013). Zhu et al. (2021) investigated the seasonal dynamics of phytoncide emissions across five forest stands: *P. edulis*, subtropical evergreen broad-leaved, *L. formosana*, *C. lanceolata*, and coniferous-broad-leaved mixed forests. Their findings revealed significant seasonal variations, with the highest emissions observed in summer, followed by spring, autumn, and winter. Temperature emerged as the key environmental driver, exhibiting a positive correlation with phytoncide volatilization. This can be attributed to the temperature-dependent activity of synthetase enzymes, directly influencing phytoncide production (Harley et al. 2014, Trindade et al. 2016). Light also plays a significant role, influencing plant processes like photosynthesis, transpiration, and stomatal conductance (Guenther et al. 1993). Studies have shown a direct link between light intensity and phytoncide release, with emissions increasing alongside rising light levels (Guenther et al. 1991, van Meeningen et al. 2017). This explains the observed peak in phytoncide emissions during summer, when

both temperature and sunlight are abundant. On the other hand, the monitoring results of phytoncide concentrations at different sampling sites indicate that the composition of vegetation groups not only results in differences in phytoncide composition but also impacts their concentration.

### **Features and planning recommendations for forest healing trails**

On the whole, at Lienhuachih Research Center, site A scores the top forest health index, with site B coming in second. The average concentration of phytoncides ranks in the following order: site A > site B > site C > site D. In terms of seasonal phytoncide concentrations, the ranking is listed as fall > summer > spring > winter, with both summer and fall in particular seeing the best results. Except for winter, the human comfort index scored “very comfortable” in all seasons.

Based on this study, here are some recommendations for activity planning at the forest therapy location in question. At site D where the Giant Bamboo forest is an open terrain, common practices like stretching and breathing are suggested when transitioning to site C to achieve the highest health benefits from the environment. Alternatively, activities at site D are advised to feature physiological and psychological exercises or quiet indoor pursuits. The best seasons for performing these activities are summer and fall with the highest phytoncide concentrations. On the other hand, the relatively high concentration levels of suspended particles (PM 2.5) in recent years, delivered by northeastern winds every winter, suggest winter should be avoided for running activities.

This study concentrated on phytoncides produced by trees on a small scale. However, given the rapid changes in macroscopic atmospheric models, the results of our study

are substantially influenced by large-scale weather factors. We advise subsequent regular follow-ups and monitoring to provide the best references for the optimal design of forest-based spaces and therapeutic sessions.

## CONCLUSION

This study measured seasonal variation of phytoncide composition and negative air ion concentrations at different activity sites within the Lienhuachih Research Center. The study also evaluated the human comfort index during different seasons based on environmental factors. Results of the observations indicate the following:

The human comfort index during the spring, summer, and fall seasons were classified as “very comfortable.”

1. Negative air ion concentrations increase with higher environmental humidity, ranked in the following order: summer > fall > winter > spring.
2. Of all the different sampling sites, site A exhibited the highest negative air ion concentration, while site D had the lowest concentration.
3. At each location, limonene, linalool, and terpinen-4-ol were the main phytoncide components, but their composition proportions changed with the seasons.
4. Phytoncide composition was similar between sites A and B, which recorded higher concentrations than sites C and D, possibly related to vegetation composition and environmental humidity.
5. The summer and fall seasons are considered optimal for planning activities at the forest therapy site.

These findings contribute to understanding the dynamic seasonal changes of phytoncide composition and air quality at the different activity sites within the Lienhuachih

Research Center, offering insights for designing and organizing practical forest healing activities.

Air quality monitored at the study was subject to unsteady airflow which disrupted data output. To obtain relatively constant results, a greater number of observation sites and sufficient data are indispensable. In addition, given the limits of the study period and budget, we could only achieve a preliminary analysis, calculating 3-year averages based on survey results. The environmental idiosyncrasies of the therapy site could be better understood with data collected over many more years. Moreover, in light of the comfort levels derived from phytoncides, this research also recommends further cross-analyses studying the therapy sites and designing timelines for sessions supplemented by effectiveness for therapy participants to improve evidence-based forest healing practices.

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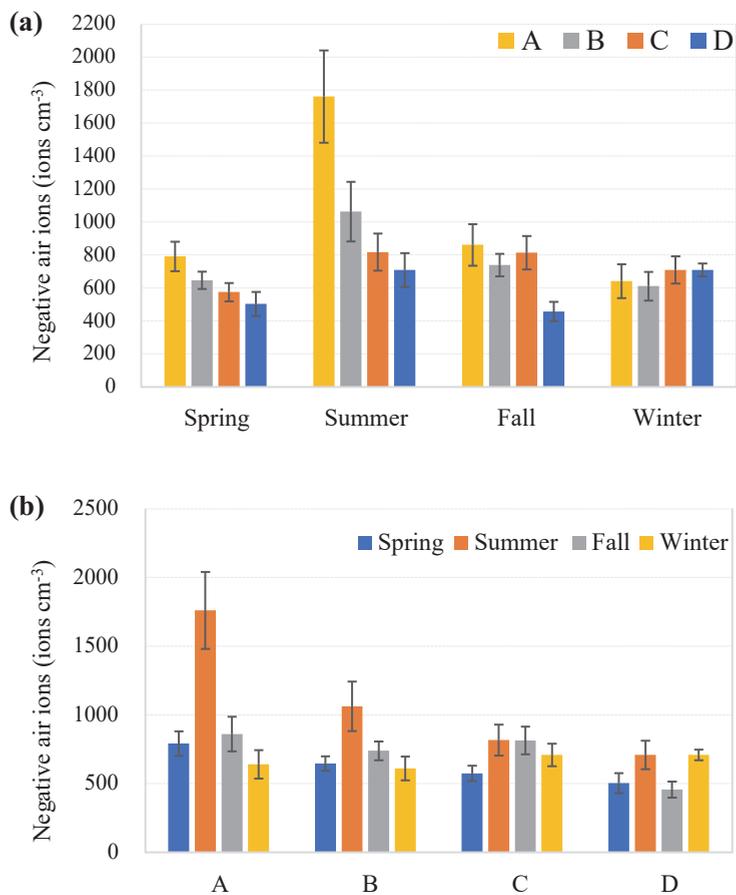
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**Fig. S1.** NAI concentrations across different seasons (a) and sampling sites (b)