**Title** Natural volatile organic compounds (NVOCs) are greater and more diverse in UK forests compared with a public garden

**Running head** Natural volatile organic compounds in UK forests

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**Abstract**

Forest bathing is based upon a Japanese practice known as Shinrin-Yoku and is a nature-based therapy involving mindful walks through ancient woodland to reduce stress and anxiety. One proposed mechanism behind the effectiveness of Forest bathing is based on the potential mental and physical health benefits of the natural volatile organic compounds (NVOCs) that fill the forest understory. All plants emit NVOCs, which play important functions in stress tolerance and communication with other organisms; however, we know surprisingly little about the concentrations and diversity of plant NVOCs in ambient air particularly in the UK. Mass spectrometry is a highly sensitive technique for measuring chemical compounds and is ideal for the analysis of the chemical composition of the forest air. This study aims to increase the knowledge of the NVOC’s within a UK forest environment. Air samples were collected in July 2022 in a UK forest and compared with samples from a walled garden environment. The samples were collected over a 2-hour time period and analysed using GC-MS and clear differences in the chemical composition of the air could be seen in the 2 environments using principal component analysis (PCA). This study revealed NVOC’s including limonene, carvone, terpenes, terpenoids and sesquiterpenoids were present within a UK forest but were either not all present or present at little to low levels in the control setting of a walled garden. This study also found that the typical 2hr duration of a Forest bathing session was a long enough sampling period to detect these NVOC’s, indicating that Forest bathers could benefit from exposure to NVOC’s.

**Key words:** Forest; Forest bathing; Mass spectrometry; NVOC’s; Phytoncides;

**Introduction**

The COVID-19 pandemic resulted in 46% of UK adults spending more time outside, with 40% commenting that nature was more important to their well-being than before the pandemic [1]. The mental health and well-being advantages of being out in nature are already well proven [2, 3] however, often these studies are observational or cross-sectional and are not always scientific based research such as public opinion surveys conducted by non-governmental organisation’s. In addition, although spending time in nature is beneficial, greater health and well being benefits can be found from more active engagement with nature or ‘nature connection’ activities such as Forest bathing.

Forest bathing is based upon a Japanese practice known as Shinrin-Yoku and is a nature-based therapy involving mindful walks through ancient woodland to reduce stress and anxiety [2]. Forest bathing was introduced as a National prescription in Japan to tackle work-stress. There is a wealth of research from Japan and South Korea which evidences the effectiveness of Forest bathing (compared with spending time in urban settings), in improving health and wellbeing outcomes [3, 4]. Japanese and South Korean researchers have consistently measured the physiological outcomes of Forest bathing and found improvements in blood pressure [5] , immune function [6] , pro-inflammatory cytokines (e.g. cortisol) [7] and cardiovascular health [8].

One of the proposed mechanisms for these physiological improvements, is suggested to be the presence of natural volatile organic compounds (NVOC’s ) found in the forest. All plants emit NVOCs, which play important functions in the plants stress tolerance and communication with other organisms. However, the defensive properties of NVOC’s are also thought to impact human physiological defence systems. Hence, Forest bathing researchers in Japan have examined the effect of NVOC’s on the human immune system. In a controlled experimental design by Li et al [9] a significant increase in Natural Killer (NK) cells and different anti-cancer proteins was found from participants blood samples after they engaged with a three-day Forest bathing retreat compared with a trip to the city. The VOC’s alpha and beta pinene were found at the study location in the forest but not in the city in Tokyo. They also found that when NK cells were incubated with VOC’s over five days, NK cells and anti-cancer proteins increased. These immune system benefits lasted for more than 7 days but started to return gradually to baseline after 30 day which led to recommendations that Forest bathing should be practiced for 2-3 hours once a month to maintain the beneficial effects on the immune system [10]. In a further study to simulate the forest environment Li et al diffused VOC’s (hinoki cypress) into participants hotel rooms over three nights and replicated the previous findings of increased NK cells and anti-cancer proteins from blood samples (T cells, and granulysin, perforin, granzyme A/B-expressing lymphocytes) [11]. They also found evidence of psychosocial improvements including increased sleep duration, reduced stress hormones (adrenaline and noradrenaline), and reduced stress.

In addition to examining the effects of VOC’s on the human immune system, and finding a 50% increase in human NK cells following three days of Forest bathing, Li et al [6] established the presence of VOC’s in the forest and found VOC’s (such as alpha-pinene, beta-pinene and isoprene) were present in the forest but not in urban settings. In more recent studies, researchers have examined VOC levels in different types of natural setting and amongst different predominant woodland species. For example, in 2015 Lee et al [12] found a higher concentration of VOC’s in the forest compared with an arboretum. Consistent with this finding, in 2018 Lee et al. [13] reported that VOC’s were higher in a natural than a tended forest. Sampling locations and species present therefore influence the levels and types of VOC’s found with some research [14] finding that conifer trees have a higher concentration of VOC’s compared to deciduous trees.

However little research has been conducted to measure actual levels of NVOC’s within a forest environment and studies often involve different types of woodland. Very little research has been done on the chemical composition of forest air in Europe or the UK with the only study on European forests (Iberian Peninsula, Spain) by Bach et al [15] finding that alpha-pinene was the monoterpene found with the highest concentration. However, Bach et al also argued that owing to the lack of forest descriptions in 19.35% of papers reviewed, it was also difficult to ascertain which characteristics of woodland and which tree species, offered the greatest levels of VOC’s [15]. Therefore, currently it is unclear whether the same beneficial volatile chemicals are present in a UK forest as in Japanese and South Korean forests.

The total area of woodland in the UK is estimated to be around 3.24 million hectares as at March 2022. This represents around 13% of the total land area in the UK [17]. However, the total area of ancient woodland is estimated to be around 2.5% of the total land area of the UK [18]. Although ancient woodlands represent a small area of the UK they are very important habitats. Ancient woodlands are classified as either ancient semi-natural woods, which have had woodland cover for over 400 years, or as plantations on ancient woodland sites, which have been replanted with non-native species. What makes ancient woodland sites so special is that each one is unique and houses a complex mix of biodiversity which has accumulated over hundreds of years. These are special and unique environments which give off a wide range of volatile chemicals.

Previous studies have identified a group of compounds often referred to as phytoncides as one of the main drivers of the benefits of Forest bathing. The term phytoncide is derived from ancient Greek and relates to the antimicrobial and insecticidal activity of these particular compounds. These compounds are often monoterpenes such as alpha- and beta- pinene and limonene however the more general term NVOC’s covers all naturally occurring volatile compounds and includes other classes of compounds such as sesquiterpenes alongside many other low molecular weight compounds under 400Da in mass. As this study combined an untargeted global analysis approach alongside more targeted approaches the term NVOC’s is used to describe the compounds measured.

The plant kingdom contains over 200,000 metabolites and over 1000 of these are NVOC’s which are emitted by plants and trees. These compounds are readily analysed using GC-MS and standard methods of collection include collection onto thermal desorption tubes or charcoal traps. These methods allow for the collection of multiple NVOC’s and are stable so can be collected in the field and analysed later.

The bulk of previous work in this area has involved collecting samples for around 24 hours which would not be comparable to the exposure of the body due to the average length of time that a forest-bather would spend in the forest environment (2hrs). This study is aimed at not only collecting information on the chemical composition of forest air in a semi-ancient woodland in the UK but also on drastically reducing the length of time taken to collect air samples to give a more comparable estimate of the exposure of the human body to these VOC’s.*Aims*

This study aimed to increase the knowledge of the NVOC’s within a UK forest environment and to assess what exposure to NVOC’s a typical forest bather might have. These aims will be assessed by collecting air samples over 2hrs in a UK ancient woodland and comparing these forest samples with samples from a walled garden environment. We hypothesise that greater levels and a more diverse composition of NVOC’s will be found in ancient woodland, compared with a managed walled garden.

Materials and Methods

Sampling Site

A sampling and control site were chosen and marked using the what3words app to get a detailed geographical location. The control site was a walled garden within the grounds of the University of Sheffield and the forest site was an area of ancient woodland known as Ecclesall Woods within the city boundary of Sheffield, South Yorkshire, UK. The area is the largest ancient woodland in South Yorkshire, UK covering an area close to 140 hectares with a mix of species mainly oak and birch but also including alder, ash, elm, field maple, hawthorn, hazel and rowan, which all occur naturally. Other non-native species were introduced to the woods in the early 19th century including beech, hornbeam, larch, Scot’s pine, sweet chestnut and sycamore. The walled garden was chosen as a convenient, controlled outdoor space which contained flowerbeds, a grassed area and trees and also allowed for sampling away from any traffic related volatile chemicals in the air. The composition of species with a 5 metre radius of both sampling sites was noted and this is shown in Table 1. Samples were all collected in July 2022 between the hours of 10am and 1pm within a temperature range of 20-22oC and humidity range of 59-65%.

|  |  |
| --- | --- |
| **Tree Species in Forest** | **Tree Species in Walled Garden** |
| Oak | Maple |
| Beech | Willow |
| Rowan |   |
| Holly |   |
| **Ground Cover in Forest**  | **Ground Cover in Walled Garden** |
| Grass | Grass |
| Brambles | Lavender |
| Thistle |   |
| Oak saplings |   |
| Holly saplings |   |
| Sweet chestnut saplings |   |
| Bracken |   |

Table 1: Tree and ground cover species found within 5m radius of air sampling site.

Collection of samples

A BUCK Elite™ model Elite-1 Personal Air Sampler was used to pump air through Stainless Steel Tenax GR 60/80 Packed thermal desorption (TD) Sample Tubes (Perkin Elmer). The tubes were all pre-conditioned at a temperature of 300oC and a flow rate of 50ml/min helium. The pump was connected to a manifold allowing collection onto 4 tubes simultaneously. The pump was mounted at a height of 1.5m above the forest floor using a camera tripod see figure 1 for set-up of equipment. The flow rate of sampling was 500ml/min and air was sampled for 2 hours. As this was a preliminary study only a small number of samples were taken: 4 samples were collected in the forest and 4 samples were collected in the control walled garden area. The tubes were removed from the manifold and end capped immediately after sampling. These tubes were then loaded within 24 hours onto a Perkin Elmer Turbo Matrix 650 thermal desorption unit attached to a Perkin Elmer Clarus SQ8T GC-MS system. The temperature ramp for desorption was 40oC/sec up to a final temperature of 290oC with a flow rate of 50ml/min of helium. The tubes underwent a 2-stage desorption of 5 minutes followed by 2 minutes. The samples were injected into the GC-MS onto a Perkin Elmer MS-5 capillary column. The initial oven temperature was 50oC and the temperature of the GC oven was ramped at 20oC per minute to a final temperature of 230oC and held there for 10 minutes. Data was collected over the mass range 50-400amu.



Figure 1 Collection of air sample set-up of tripod and pump in the forest.

Data Analysis

The data was converted to the global mzML format and processed using XCMS Online Scripps research tool [19] for processing metabolomic data. The tabulated output from this was imported into SIMCA 17 (Umetrics) and principal component analysis (PCA) plots were produced to understand the overall differences in the air samples of the forest environment vs the walled garden. Phytoncide compounds present in the samples were identified using the NIST library.

Results

The results of the PCA analysis are shown in figure 2. PCA scores plots are commonly used to look at global changes in sample composition therefore are a good indicator of differences between sample types without identification of particular compounds. In this case 2 components explained more than 70% of the variance in the 2 sample groups and a clear difference is seen in the overall make of the air in the 2 different environments. There was good reproducibility found between the replicate samples which gave confidence in the sampling method and the reduced time for sample collection.

Figure 2 PCA plot of the global metabolic profile of the two different air types. Forest air vs control air.

A PCA allows an untargeted approach to data analysis however the presence of particular compounds including terpenes and terpenoids was also of interest to this study. The forest air data was mined for these compounds which were identified using the NIST library. The most abundant compounds found in the forest air which were not present in the control air samples are listed in Table 2.

|  |
| --- |
| **Chemical Name**  |
| Nonynoic Acid |
| Benzene |
| Toluene |
| Benzaldehyde |
| Phenol |
| Octanal |
| Limonene |
| Acetophenone |
| Nonanal |
| Benzoic Acid |
| Decanal |
| Carvone |
| Phthalic Acid |
| Hexadecanal |
| γ-Elemene |
| Levomenthol |

Table 2: List of the most common NVOC’s found in the forest air samples which were not present in the air samples.

Of particular interest in the Table are limonene, carvone and γ-elemene which are a monoterpene, a monoterpenoid and a sesquiterpenoid respectively. These compound classes have all been found previously in forest air and have been linked to the benefits of Forest bathing. Previous studies identified both alpha- and beta- pinene as major components within forest air, which were not identified in this study [12, 15]. However, these previous studies have not been performed within a UK ancient woodland hence the NVOC mixture within a UK forest is unknown. The timing of collection of samples has been shown to affect the levels of NVOC’s found in the forest. The air samples in this study were all collected in late morning between 10am and 1pm hence this may have affected the range of compounds identified. Previous research has shown that levels of phytoncides vary according to time of day with levels peaking in the early afternoon especially in the summertime but also even in the early morning [14]. The timing of collection in this study however was convenient for when the forest, a popular area for recreation, was quieter and therefore suitable for Forest bathing. Levels of phytoncides can also be affected by several other factors including meteorological conditions, altitude, seasons, sunlight exposure and tree species [20]. Tree species in particular have been shown to affect levels as it has been demonstrated that those forests consisting mainly of conifer trees have a higher concentration of NVOC’s than those forests consisting of more deciduous trees [14]. In this study it is also possible that the short sampling collection time has reduced the number of compounds found however, as this length of time is more comparable to the length of time spent Forest bathing it is perhaps more representative of human exposure to NVOC’s with Forest bathing which usually lasts between 2-4 hours and a generally recommended time of around 120 minutes to spend in nature each week being beneficial to well-being [21].

Although this was only a small study it clearly showed the presence of some NVOC’s including the presence of terpenes, terpenoids and sesquiterpenoids within a UK forest. It also proved that even with a relatively short sampling time these NVOC’s can be detected and therefore potentially can have an effect on the human body with only a short time of exposure to them. The control environment garden showed little to low levels of these NVOC’s showing that they are specific to a forest area particularly as the forest cover could lead to a concentration of these chemicals as opposed to the open walled garden area. Some of the common NVOC’s were not detected in the samples and this could be down to the low concentration of these particular NVOC’s in the forest air and so with a shorter sampling time these were not detected. Samples were collected between the hours of 10am and 1pm which is slightly earlier than the optimum time of early afternoon. Again, this could affect the levels measured however it must not be forgotten that the benefits of Forest bathing are not limited to just the presence of NVOC’s but also to the forest environment sights and sounds and the relaxation and mindful techniques which go hand in hand with Forest bathing.

This initial preliminary study shows clear differences in the composition of UK ancient woodland air as opposed to a walled garden outdoor environment. It allowed the identification of some common NVOC’s with the forest air which were not detected in the control environment. It also revealed that exposure for 2hrs, the typical duration of a Forest bathing session, would be sufficient time for humans to be exposed to NVOC’s and potentially benefit from them in terms of NVOC’s immune-regulating properties. Cross-sectional studies in Italian forested areas suggest potential immuno-protection from evergreen Mediterranean forests and shrubland and found associations between the presence of forests and lower COVID‑19 mortality [22]. There is a need to go beyond correlational studies and to conduct further research taking samples of NVOC’s from forested areas and measuring the immune status of the people living close to them and spending time in them. To progress this study further and understand more about the chemical composition of UK forest air additional samples need to be collected and compared with control sites such as managed forestry. Comparisons of NVOC’s found in different forest types have indicated that a mix of coniferous and broadleaved woodland are often healthier and emit more NVO’s and oxygen [23]. A comparison of NVOC’s found in different forest types across the UK could inform reforestation planting schemes, bringing optimal benefits to forest and human health. The UK Government has proposed tree planting to respond to the climate crisis [24], however, if greater levels and diversity of NVOC’s can be found in mixed ancient woodland, more natural processes such as protection of existing ancient woodland and natural woodland recovery and recolonization (succession) could result in healthier woodlands and healthier humans. Such research could build the argument for protection of ancient woodland as a preventative health resource and promote natural reforestation of areas in the UK.

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