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Natural Volatile Organic Compounds (NVOCs) Are Greater and More Diverse in UK Forests Compared with a Public Garden

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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. Surprisingly little is known about the concentrations and diversity of plant NVOCs in ambient air particularly in the UK and this study aims to increase that knowledge. 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 h time period and analysed using GC-MS and showed clear differences in the chemical composition of the air. This study revealed NVOCs including limonene, carvone, terpenes, terpenoids and sesquiterpenoids were present within a UK forest but were either not present or present at little to low levels in the control setting of a walled garden. This study also found that the typical 2 h duration of a Forest bathing session was a long enough sampling period to detect these NVOCs, indicating that Forest bathers could benefit from exposure to NVOCs.

Keywords: forest; forest bathing; mass spectrometry; NVOC's; phytoncides

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1. 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, for instance a public opinion survey conducted by non-governmental organisations. 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 provides evidence for 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 (NVOCs) found in the forest [9]. Regardless of the type of forest all plants and trees can synthesise and emit NVOCs, through processes generally triggered by abiotic and biotic stress factors and these same NVOCs are also thought to impact human physiological defence systems. However, tree and forest air can have a wider impact on air quality as well as human health by aiding pollution removal [10]. Forest bathing researchers in Japan have examined the effect of NVOCs on the human immune system. In a controlled experimental design by Li et al. [11], a significant increase in Natural Killer (NK) cells and anti-cancer proteins was found in participants' blood samples after they engaged in a three-day Forest bathing retreat compared with a trip to Tokyo city. The NVOC's alpha and beta-pinene were found at the study location in the forest but not in the city. They also found that when NK cells were incubated with NVOCs 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 days which led to recommendations that Forest bathing should be practiced for 2–3 h once a month to maintain the beneficial effects on the immune system [12]. In a further study to simulate the forest environment, Li et al. diffused NVOCs from hinoki cypress (*Chamaecyparis obtusa*) 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) [13]. They also found evidence of psychosocial improvements including increased sleep duration and reduced stress levels along with a reduction in stress hormones (adrenaline and noradrenaline).

In addition to examining the effects of NVOCs 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 NVOCs in the forest and found NVOCs (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 NVOC levels in different types of natural settings and amongst different predominant woodland species. For example, in 2015 Lee et al. [14] found a higher concentration of NVOCs in the forest compared with an arboretum. Consistent with this finding, in 2018 Lee et al. [15] reported that NVOCs were higher in a natural forest rather than a tended one and in 2019 Meneguzzo et al. [16] found that conifer trees produce a higher concentration of NVOCs compared to deciduous trees. Therefore, sampling location and the tree species present highly influence the levels and types of NVOCs found in the forest environment.

Very little research has been conducted on the chemical composition of mixed deciduous ancient woodland air in Europe or the UK with one of the few studies on a deciduous European forest (Iberian Peninsula, Spain) by Bach et al. [17] finding that alpha-pinene was the monoterpene found with the highest concentration. Other European studies include a study on a boreal Scot's Pine (*Pinus sylvestris*) dominated forest in Finland which found that alpha-pinene was the most abundant monoterpene [18] and also a study on the variability of terpenes in conifers which found the terpene profile to be different within different species of conifers (*Pinus* sp., *Picea* sp., and *Abies* sp.) [19]. Another recent study in a coniferous forest (*Picea* sp. and *Abies* sp.) in Serbia found alpha-cadinol and spathulenol as the most abundant terpene species [20]. However, Bach Pagès 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 gave rise to the greatest levels of NVOCs [21]. Therefore, currently it is unclear whether the same beneficial volatile chemicals are present in a UK mixed deciduous forest as in other types of forests around the world.

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 [22]. However, the total area of ancient woodland is estimated to be around 2.5% of the total land area of the UK [23]. 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 individual environments which emit 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 [9]. These compounds are volatile organic compounds (VOCS) and are often monoterpenes such as alpha- and beta pinene and limonene however the term NVOCs 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 NVOCs is used to describe the compounds measured.

The plant kingdom contains well over 200,000 metabolites [24] and around 1700 of these are NVOCs which are emitted by plants and trees [25]. These compounds are readily analysed using GC-MS (gas chromatography–mass spectrometry) and standard methods of their collection is absorption onto thermal desorption (TD) tubes or charcoal traps. These methods allow for the collection of multiple NVOCs and are stable so can be collected in the field and analysed later.

The aim of this work is to collect samples of forest air to compare to the exposure of the body to NVOCs in a typical Forest bathing venture into the forest. This study also aims to analyse the chemical composition of a UK semi-ancient woodland within a 2 h sampling period to give a comparable estimate of the exposure of the human body to these NVOCs. Currently there is very little information about the chemical composition of UK forest air and as Forest bathing becomes more popular in the UK this type of information will help to identify the type of forests most likely to provide higher concentrations of beneficial NVOCs. This in turn may dictate the future of Forest bathing and forest-based therapeutic sessions within the UK.

Overall, this is a preliminary study to increase the knowledge of the NVOC's within a UK semi-ancient, deciduous 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 2 h in a UK ancient woodland and comparing these forest air samples with samples from a walled garden environment.

2. Materials and Methods

2.1. Sampling Site

A sampling and control site were chosen and marked using the what3words app (https://what3words.com/ (accessed on 23 December 2022)) to obtain detailed geographical locations. 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 (see Figure 1). The area is a mixed deciduous woodland in South Yorkshire, UK covering an area close to 140 hectares with a mix of tree species mainly oak (*Quercus robur*) and birch (*Betula pubescens*) but also including alder (*Alnus glutinosa*), ash (*Fraxinus excelsior*), elm (*Ulmus procera*), field maple (*Acer campestre*), hawthorn (*Crataegus laevigata*), hazel (*Corylus avellana)* and rowan (*Sorbus aucuparia*), which all occur naturally. Other non-native species were introduced to the woods in the early 19th century including beech (*Fagus sylvatica*), hornbeam (*Carpinus betulus*), larch (*Larix decidua*), Scot's pine (*Pinus sylvestris*), sweet chestnut (*Castanea sativa*) and sycamore (*Acer pseudoplatanus*). The walled garden was chosen as a convenient, controlled outdoor space which contained flowerbeds of lavender (*Lavandula angustifolia)* and pansies (*Viola tricolor hortensis*), a lawned 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 10 am and 1 pm within a temperature range of 20–22 °C and humidity range of 59%–65%.

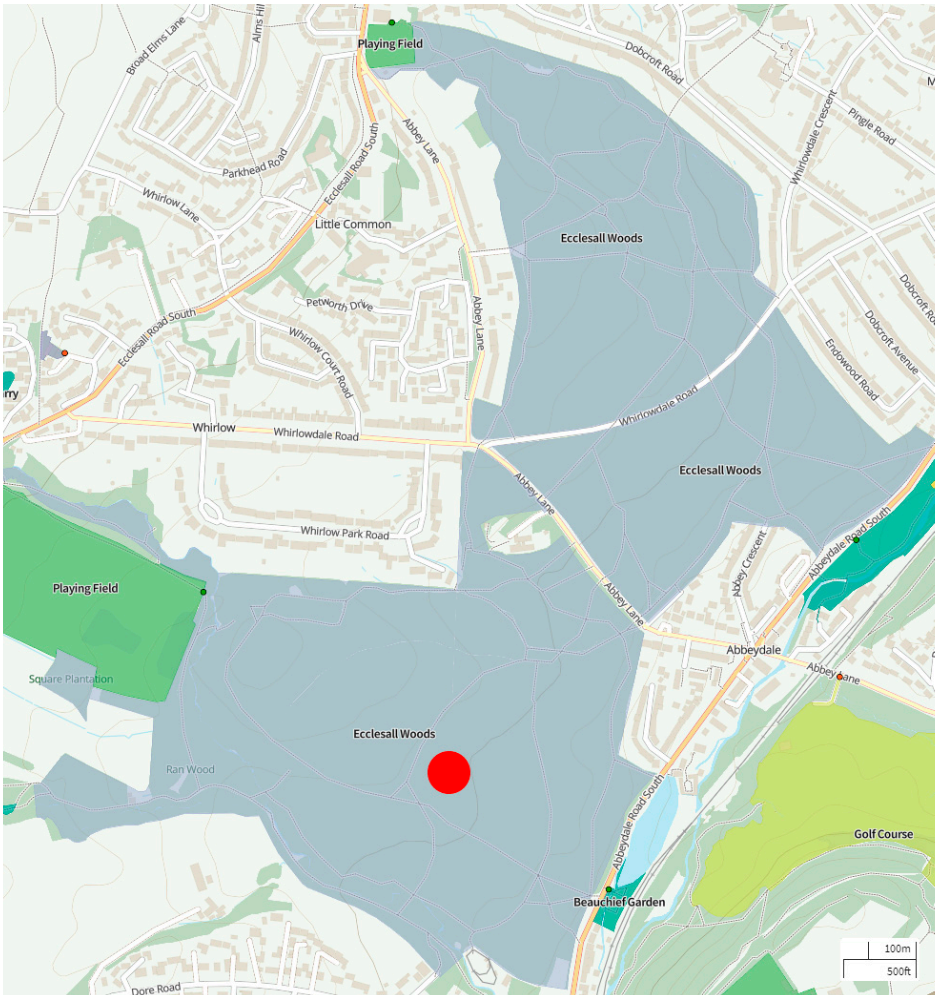

Figure 1. Map of sampling site in Ecclesall Woods. Red dot marks sampling site. Map courtesy of Ordnance Survey.

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

Tree Species in Forest	Tree Species in Walled Garden
Quercus robur	*Acer platanoides*
Fagus sylvatica	*Salix alba*
Sorbus aucuparia	
Ilex aquifolium	
Ground Cover in Forest	Ground Cover in Walled Garden
Poaceae (mixed grasses)	*Poa annua*
Rubus allegheniensis	*Lavandula angustifolia*
Cirsium vulgare	*Viola tricolor hortensis*
Quercus robur saplings	
Ilex aquifolium saplings	
Castanea sativa saplings	
Pteridium aquilinum	

2.2. Collection of Samples

Air samples were collected using a BUCK Elite™ model Elite-1 Personal Air Sampler pump to pump air through Stainless Steel Tenax GR 60/80 packed thermal desorption (TD) sample tubes (Perkin Elmer). The tubes were packed with a silica-based packing material onto which NVOCs were absorbed. The tubes were all pre-conditioned at a temperature of 300 °C and a flow rate of 50 mL/min helium. The pump was connected to a manifold allowing collection onto 4 tubes simultaneously. The pump was mounted at a height of 1.5 m above the forest floor using a camera tripod, see Figure 2 for set-up of equipment. The flow rate of sampling was 500 mL/min and air was sampled for 2 h. This is because the average length of time that a forest-bather would spend in the forest environment is 2 h. 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.

Figure 2. Collection of air sample set-up of tripod and pump in the forest.

2.3. Sample Analysis

The TD tubes were then loaded within 24 h onto a Perkin Elmer Turbo Matrix 650 thermal desorption unit attached to a Perkin Elmer Clarus SQ8T GC-MS (gas chromatograph mass spectrometer) system. The thermal desorption unit used a temperature ramp to desorb the NVOCs into the GC-MS system. The temperature ramp for desorption was 40 °C/sec up to a final temperature of 290 °C with a flow rate of 50 mL/min of helium. The tubes underwent a 2-stage desorption of 5 min followed by 2 min. The samples were injected into the GC-MS and onto a Perkin Elmer MS-5 capillary column (30 m, id 0.25 mm) to separate out the compounds. GC-MS is a well-characterized analytical technique for the measurement and identification of volatile organic chemicals. The initial oven temperature was 50 °C and the temperature of the GC oven was ramped at 20 °C per minute to a final temperature of 230 °C and held there for 10 min. Data were collected in electron ionization (EI) mode over the mass range of 50–400 amu.

2.4. Data Analysis

The GC-MS spectra were converted to the global mzML format and processed using XCMS Online Scripps research tool [26] for processing metabolomic data. The tabulated output of mass, retention time and intensity value from this process was imported into SIMCA 17 (Umetrics) and principal component analysis (PCA) plots were produced to understand the global differences in the air samples of the forest environment vs. the walled garden without targeting any specific compounds. NVOC compounds present in the samples were identified using the NIST library.

3. Results

The results of the PCA analysis are shown in Figure 3. PCA scores plots are commonly used to look at global changes in sample composition, therefore, are good indicators of differences between sample types without the identification of particular compounds. In this case, 2 principal components explained more than 70% of the variance in the 2 sample groups and a clear separation between the 2 air groups was seen in the overall make up of the air in the 2 different environments. This shows that there was good reproducibility between the replicate samples which gave confidence in the sampling method and the short time for sample collection.

Figure 3. PCA plot of the global NVOC profile of the two different air types. Forest air vs. control air from the walled garden.

The identity of compounds which were different between forest air and air from the walled garden was also of interest to this study. Using the PCA analysis the forest air chromatograms were mined for the compounds which were different between the forest air and the control walled garden air and these compounds were identified using the NIST library. Some of the most abundant compounds which were different between the two air types are listed in Table 2 along with their relative abundances.

Table 2. List of the most common NVOCs found in the forest air samples. N/D denotes compound not detected.

Compound Name	Relative Abundance Forest Air %	Relative Abundance Control Walled Garden %
Acetophenone	0.81	0.54
Benzaldehyde	ND	0.02
Benzene	1.37	0.82
Benzoic Acid	5.12	8.23
Carvone	0.06	N/D
Decanal	0.68	0.41
γ-Elemene	0.05	N/D
Levomenthol	0.20	0.12
Limonene	0.07	N/D
Nonanal	0.76	0.46
Octanal	0.46	0.27
Phenol	0.72	1.69
Toluene	0.71	0.68

4. Discussion

Compounds of particular interest in Table 2 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 beneficial health traits. Limonene in particular has been found to have many health benefits such as antioxidant, anti-inflammatory, anticancer, antinociceptive and gastroprotective characteristics [27]. There are also several studies showing limonene to have a neuroprotective role, particularly in cases of anxiety and depressive-like behaviour [28,29]. Carvone has been found to exhibit many pharmacological properties such as antibacterial, antifungal, antiparasitic, anti-neuraminidase, antioxidant, anti-inflammatory, and anticancer activities [30] and elemene has been used in Chinese medicine to treat several types of cancer [31]. Other compounds of note which were detected are the benzenoid compounds (benzene, benzoic acid, toluene and phenol) which are commonly emitted from vegetation and which also have been shown to have various health benefits as antioxidants [32]. Previous studies on NVOC's in forest air have identified both alpha- and beta-pinene as major components within forest air, which were not identified in this study [14,17]. However, these previous studies were not performed within a UK ancient mixed deciduous woodland therefore the NVOC mixture within a UK forest is unclear. The timing of the collection of samples has been shown to affect the levels of NVOCs found in the forest. The air samples in this study were all collected in late morning between 10 am and 1 pm hence this may have affected the range of compounds identified. Previous research has shown that levels of NVOCs vary according to time of day with levels peaking in the early afternoon, especially in the summertime but also in the early morning [16]. 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 the collection of samples and also for Forest bathing. Levels of NVOCs can also be affected by several other factors including meteorological conditions, altitude, seasons, sunlight exposure and tree species [9]. 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 NVOCs than those forests consisting of more deciduous trees [16]. It is also possible that the short sampling collection time used 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 NVOCs as a Forest bathing session usually lasts between 2-4 h and there is a generally recommended time of around 120 min to spend in nature each week being beneficial to well-being [33].

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 NVOCs found in different forest types have indicated that a mix of coniferous and broadleaved woodland are often healthier and emit more NVOCs and oxygen [34]. A comparison of NVOCs 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 [35], however, if greater levels and diversity of NVOCs 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.

Although this was only a small study it clearly showed the presence of some NVOCs including the presence of terpenes, terpenoids and sesquiterpenoids with known health benefits within a UK forest. It also proved that even with a relatively short sampling time these NVOCs can be detected and therefore potentially have an effect on the human body with only a short time of exposure to them. There were some limitations to the study as discussed above however it must not be forgotten that the benefits of Forest bathing are not limited to just the presence of NVOCs but also to the forest environment sights and sounds and the relaxation and mindful techniques which go hand in hand with Forest bathing.

5. Conclusions

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 NVOCs within the forest air which were not detected in the control environment. A wide range of NVOCs were identified in the forest air including common terpenoids. This type of study has not been performed in a UK semi-ancient, deciduous forest previously and helps to understand the NVOC air composition within this environment. The study also revealed that exposure for 2 h, the typical duration of a Forest bathing session, would be sufficient time for humans to be exposed to NVOCs and potentially benefit from them in terms of NVOCs immune-regulating properties.

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