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# Phenolic compounds in Scots pine heartwood: are kelo trees a unique woody substrate?

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## Abstract

Deadwood quality can be a highly significant factor in determining the occurrence of deadwood-dependent organisms such as saproxylic fungi. Rare deadwood substrates that are produced only after a lengthy senescence, such as kelo trees, may have unique deadwood qualities. Using high-performance liquid chromatography (HPLC), we compared the phenolic composition of six types of Scots pine substrates; living mature trees with no fungal sporocarps, living mature trees with *Phellinus pini* sporocarps, fallen non-kelo trees, soon-to-be-kelo (standing), standing kelo and fallen kelo. The fungal infected living trees and fallen kelos were found to have more similarities in their phenolic composition when compared to the living and fallen trees and the standing kelos, which gets further pronounced with increasing decay. The results also highlight the uniqueness of the fungal infected living trees and the fallen kelos and illustrate a possible correlation between fungal infection and the heartwood phenolic composition of Scots pine. However, it remains unclear to what extent the differences in phenolic compositions are caused by fungal infection and fungal decomposition. We also observed a previously undocumented correlation between the phenolic groups and fire scars on the trunks of the trees. The variation in substrate quality warrants further consideration when deadwood restoration activities are planned, as the quality of the deadwood could be as equally important as the quantity.


**Key-words:** boreal forest, chemical diversity, deadwood, fungal decay, heartwood phenolics, substrate quality, tree resistance

## Introduction

Forest deadwood substrates, comprised mostly of snags, fallen dead trunks and stumps (Hagemann et al. 2009), are of high ecological value as they maintain a large part of the structural and biological diversity of forests (Tikkanen et al. 2006; Hottola et al. 2009). They provide a habitat for numerous organisms and act as a long-term nutrient source for soil detritivores (Siitonen 2001). In particular, old growth forests provide a high volume of very diverse deadwood types (Siitonen 2001), some of which are rare and structurally or morphologically unique with characteristic associated species (Niemelä et al. 1995; Renvall 1995; Niemelä et al. 2002).

Large, standing pine trees (*Pinus sylvestris* L.) and pine snags are common in natural old-growth pine forests but rarities in managed forests throughout boreal Europe. These trees have characteristic growth and death patterns (Niemelä et al. 2002). Typically, they lose their growth vigor slowly and die gradually while still standing. The death of large pines may take centuries (Rouvinen et al. 2002). During this process, the sapwood is colonized by blue-stain fungi, giving these decorticated trees a silvery grey color on the debarked surface. These trees are called '*kelo'* in the Finnish language, and the usage of the word has been extended to English texts to describe similar kinds of trees. The blue-stain fungi utilize the cell contents, leaving the lignin, cellulose or hemicelluloses undecayed thereby causing no true decay but making the wood unattractive for most decay-causing fungi (Niemelä et al. 2002).

In addition to the silvery-grey debarked trunk, standing kelos are characterized by the absence of the whole crown from the standing trunk or by the presence of just a few thick branches. In most old kelos, the sapwood has decayed and worn away leaving only the

eroded heartwood. Kelos may stay standing for several decades or even a few centuries (Rouvinen et al. 2002). Old kelos have almost always experienced at least one fire episode during their 'growth and death' period, sometimes resulting in a charred surface, either on the outer surface or in the inner layers of the heartwood, depending on whether the fire occurred during or after the death process. Niemelä et al. (2002) suggested that fungal succession, fungal species diversity and the rate of decomposition are fundamentally different in kelos than in old pine trees which fall while still alive, for example, during windstorms. Standing trees decompose more slowly than fallen ones because their trunk is not in contact with the soil and, consequently, the moisture content in the woody tissues is much lower (Yatskov et al. 2003).

Kelos have considerable economic and ecological value. Although Scots pine is one of the most common trees in this region (Palviainen et al. 2010), kelos are considered a 'vanishing' substrate and a very slowly renewable woody material in north European boreal forests (Niemelä et al. 2002). This is partly due to the slow formation of kelos, which results in approximately one kelo tree per hectare in a decade (Rouvinen et al. 2002). In addition, kelos are considered as an excellent and expensive raw material for buildings as a result of their resistance to decay. An in-depth understanding of the chemical constitution of kelos, in particular the phenolic characteristics, would be highly beneficial when seeking to increase the natural durability of the wood to wood rot. From an ecological viewpoint, decay resistance affects deadwood dynamics and, therefore, carbon and nutrient dynamics in forest ecosystems for example. However, to the best of our knowledge, no attempts have been made to analyze the chemical constitution of the kelos in comparison to live or dead non-kelo pine trees.

Wood extractives that reside in the lignocellulosic woody tissue consist of several chemical components, such as triglycerides, steryl esters, resin acids, free fatty acids, sterols and phenolic compounds, such as terpenoids, flavonoids and tannins. These compounds are considered as significant factors in contributing to the natural decay resistance of the heartwood (Hart and Shrimpton 1979; Heijari et al. 2005; Ekeberg et al. 2006). The extractive concentration is found to vary between tree species, between individual trees within the same species and also among growth rings of an individual tree. Studies have suggested a correlation between tree growth vigor (in terms of annual growth ring width) and heartwood extractive content (Taylor et al. 2003).

Recent studies have elaborated on the chemical composition of living Scots pine heartwood (e.g., Ekeberg et al. 2006), although little information is available on kelos. Scots pine heartwood predominantly contains two positively correlated phenolic secondary compounds (i.e. phenolics): pinosylvin (PS) and pinosylvin monomethyl ether (PSM) that belong to the 'stilbene' group (e.g., Harju et al. 2002, Venäläinen et al. 2004). These phenolics are known to be formed during heartwood production or as an active defence response to fungal infection (e.g., Delorme and Lieutier, 1990; Nagy et al. 2005) or injury (producing phenolics such as phytoalexins; Heijari et al. 2005). The phenolics seem to exhibit toxicity and to give low hygroscopicity to the heartwood (Celimene et al. 1999), possibly inhibiting fungal decay (Karppanen et al. 2008). However, fungal infection may also alter the capacity of the trees to produce secondary compounds (Bois et al. 1997). Furthermore, whether these fungal infection-induced changes in the phenolic content in Scots pine heartwood also hold for kelos has yet to be established. Previous studies have also found that fire scarred trees have an increased pre-disposition towards fungal attack (Geiszler et al. 1980) as a result of reduced health. Niemelä et al. (2002) have stated that kelos survive several fires in their history.

Previous studies have indicated that the concentration of total phenolics and stilbenes could be used as an indirect measure of heartwood durability against decay (Heijari et al. 2005; Harju and Venäläinen 2006). However, the presence of stilbenes alone cannot explain the defence against fungal decay, as their chemical action and mechanism still needs to be studied in detail (Hart and Shrimpton 1979; Venäläinen et al. 2004).

This study examines the variation of phenolics in the heartwood of six different types of coarse woody substrates of Scots pine, including kelo. We hypothesized that kelos are likely to be rich in secondary phenolics that are otherwise not present in live or recently killed pines because of a lengthy death process. In addition, we hypothesized that fungal attack may contribute to an alteration in the heartwood chemistry. We also hypothesized that a trend could be observed in heartwood phenolic composition during the transition of the tree from living to fungal-infected to dead, and from partially to fully dead kelo.

Although the impact of fire on the phenolic composition was considered in the analyses, as many of the sampled trees had fire scars on their trunks, it was not planned in the original sample selection and led to an asymmetrical dataset. However, we anticipated that since fires often damage trees and obviously modify the slow death process of pines, fires may also have some effect on the chemical characteristics of old pine trees and kelos.

## Materials and methods

Wood samples were obtained from the Patvinsuo National Park in Lieksa and Ilomantsi in Northern Karelia, eastern Finland ($63^0$07' N, $30^0$45'E). The region is located in the middle boreal zone. Scots pine is the dominant tree species. For this study, six different Scots pine tree types were included (listed in detail in Table 1 and Fig. 1).

5-8 trees (with a minimum diameter of 30 cm) were sampled in each of the six categories. Unequal sample numbers were unavoidable due to the low availability of trees in some categories. The trees (Table 1) were chosen randomly from the stands.

## WOOD SAMPLE EXTRACTION AND PREPARATION

Wood was collected from each substrate at 1.3 ± 0.5 m distance from the base of the tree trunk with a 5 mm increment corer. The tree diameters were measured and the corer was drilled to half of the diameter value to reach the pith. We separated the inner and outer heartwood, and the sapwood and the middle portion of heartwood was used for the study. After obtaining a core sample from the drill, the samples were immediately bagged in airtight plastic covers and sealed. The samples were transferred to the laboratory and stored at -20° C for 7-10 days. The samples were powdered, labeled and bagged in sealable covers and stored them at -20° C until further analysis. They were dried under a uniform temperature of 20° C and at 65 % humidity levels for two weeks until the moisture content became constant (15-20 %).

## EXTRACTION AND ANALYSIS OF PHENOLICS

The heartwood samples (8 mg) were milled and the powdered residue was extracted with 0.6 ml of cold methanol (100%) for 25 sec using a Precellys® 24 homogenizer (Bertin Technologies, Île-de-France, France). After standing for 15 min. in an ice bath, they were

homogenized for a second time for 25 sec and then centrifuged (Eppendorf® Centrifuge 5415R, Hamburg, Germany) at 13,000 rpm for 3 min. at +4$^{o}$ C and the methanol supernatant separated. The residue was extracted three more times using the same process, and with a reduced 5 min. standing time in the ice bath. The multiple supernatants were combined and the methanol evaporated to dryness by using an Eppendorf® concentrator (Hamburg, Germany). The dried samples were stored at -20$^{o}$ C until analyzed by the high-performance liquid chromatography (HPLC) as described by Randriamana et al. (2014). Prior to analysis, the samples were dissolved in 300 µl milli-Q water (Merck Millipore, Darmstadt, Germany): methanol (50:50, v/v), then centrifuged at 13,000 rpm for 3 minutes.

Compounds were quantified with the detector set at 220 nm. All lignans (L), neolignans (NL) and the stilbene derivatives (other than the pinosylvin group; ST-d) were quantified with the reference coefficient/Response Factor of Salicin; stilbenes (ST), including pinosylvin (PS) and pinosylvin monomethyl ether (PSM) with the reference coefficient of piceatannol; ferulic acid derivatives (FA-d) with the reference coefficient of ferulic acid; vanillic acid derivatives (VA-d) with the reference coefficient of vanillic acid; cinnamic acid derivative (CA-d) with the reference coefficient of cinnamic acid; naringenin derivatives (NG-d) with the reference coefficient of naringenin-7-glucoside; and eriodictyol derivatives (ER-d) with the reference coefficient of eriodictyol.

Mass spectrometry of UHPLC-DAD (1200 series, Agilent) equipped with a quadrupole time-of-flight mass spectrometer (Q/TOF-MS) (6340 series, Agilent) was used to identify the compounds. The Q/TOF-MS spectra collected at ESI positive ion mode with the following parameters: mass range 100–3000 *m/z*; temperature of the drying gas and sheath gas 350 °C; flow rate respectively 12-11 l min$^{-1}$; nebulizer pressure 35 psi; capillary voltage 3500 V;

nozzle voltage 1000 V; fragmentor voltage 80 V; skimmer voltage 65 V and octopole voltage 750 V. The mass-to-charge ratio value of 922.0098 was used as a reference for accurate mass measurements. The mass accuracy/error term (in ppm) was calculated based on the equation; $[M_{measured}$ (monoisotopic calculated mass) – $M_{calculated}$ (accurate mass)$\}x10^6]/ M_{calculated}$ (accurate mass).

ToF-MS provides accurate mass measurement with a mass accuracy range close to 2-5 ppm with an adequate calibration range (Lacorte and Fernandez-Alba, 2006). We calculated the monoisotopic mass of the compounds based on KEGG database (2014). The compounds identified with UPLC-DAD-MS are listed in Table 2.

STATISTICS

The differences in the mean concentration among substrates were compared using ANOVA (SPSS Version 21.0). In addition, multivariate ordination was applied using Non-Metric multi-Dimensional Scaling (NMDS) in PC-ORD Version 5.0 (McCune and Mefford, 1999) to analyze the possibility of distinct chemical groups in the six tree substrate categories.



## Results

In total, we identified 48 chemical compounds (Table 3) from the 38 pine wood samples, based on the retention times and spectral characteristics of the compounds. The main phenolic groups were stilbenes ($St_{1–10}$, including PS, PSM, resveratrol, methyl resveratrol, methyl piceatannol and one stilbene derivative (other than pinosylvin group)), neolignans ($NL_{1–7}$), lignans ($L_{1–12}$), vanillic acid derivatives ($VA\text{-}d_{1–5}$), cinnamic acid derivative (CA-d),

ferulic acid derivatives (FA-$d_{1–4}$), naringenin derivatives (NG-$d_{1–3}$) and eriodictyol derivatives (ER-$d_{1–6}$).

The stilbenes (Table 2) were identified using mass spectrometry. The high precision of the spectrometry (3.78-9.33 ppm) suggests we can accurately determine the chemical structure of secondary compounds.

Among the recorded phenolic compounds, 24 out of the 48 were found to differ between the tree types (Table 3). Different trees in the sample population exhibited different spectral chromatograms. The chromatograms of the representative trees from each tree type indicating the most important differences in the spectral signatures are presented in Fig. 3. Overlapping of the spectral absorbance peaks were noticeably more common in samples from 'living with fungi' and 'partial kelo'.



Although living trees, fallen non-kelos and the standing kelos did not exhibit significant differences in their overall phenolic chemistry (pair-wise post-hoc comparisons, Fig. 2a), noticeable differences were found in their chromatograms. Most of the trees in naturally fallen non-kelo tree group exhibited fewer phenolic compounds on the chromatogram (for example Fig. 3c), compared to the living mature trees (Fig. 3a). Concentrations of PS and PSM compounds were high in all tree types except in a few individuals of 'living with fungi' with advanced heart rot, in which an unidentified stilbene derivative ($ST_3$) showed the highest phenolic concentration (Fig. 3b). . Most of the fallen kelos (for example Fig. 3f) exhibited higher incidence of phenolics compared to the partial kelo tree samples (Fig. 3d) and standing kelos samples (Fig. 3e). There was a general similarity between the fungal infected living trees and fallen kelos in the overall phenolic structure, and in most of the specific phenolic

groups (except for VA-d, Fig. 2e). These two groups often differed from the living trees in regard to overall heartwood phenolics (Fig. 2a), as well as in all the specific phenolic groups (Figs 2 b, c, d, g, h, and i), with the exception of lignans (Fig. 2d) and CA-d (Fig. 2f).

In the NMDS analysis, the heartwood phenolic compounds concentration data of the different tree samples was run with random starting coordinates. After 94 iterations, the analyses identified a two-dimensional solution (as additional dimensions did not lower the stress value) with a final stress value 8.13878 and final instability 0.000010. No conclusive grouping was evident among the tree categories in the NMDS plot (Fig. 4). However, the fungal-infected living trees and the fallen kelos that were in the more advanced decay stage (expressed in terms of the disintegrated heartwood core) tended to be the furthest from their counterparts. Similarly, the four standing kelos that exhibited more advanced decay were also located away from the other members of this group in the NMDS plot. When the fire-scarred samples were analyzed separately, the trees with fire scars tended to separate in their overall chemical composition from the remaining trees (Fig. 5a). The phenolics; VA-$d_1$, VA-$d_4$, VA-$d_5$, $NL_1$, $NL_2$, $NL_5$, $L_1$, $L_7$, NA-$d_1$ and ST-d, were found to be associated with the grouping of the trees with fire scars (Fig. 4; Fig. 5a).

## Discussion

Despite their unique formation and attributes, heartwood from the three categories of kelos did not exhibit sufficient differences in their phenolic chemistry to establish distinct groupings from one another in the NMDS plot, or from the heartwoods of other tree groups analyzed. But decaying heartwood from fallen kelo did sort away from the other two kelo

groups, while the heartwood from fungal-infected living trees sorted away from heartwood of most other tree groups in the NMDS plot. Furthermore, the four standing kelo trees with the most advanced decay sorted more closely to the decaying fungal-infected trees and fallen kelo, whereas the four with less decayed heartwood sorted with the healthy, live trees and fallen non-kelo. This NMDS sorting of trees with advanced decay in conjunction with the overlap of living trees with the fallen healthy trees seems to imply that the dynamics involved in the decay of the latter group may be difference from the fungal-infected living trees and fallen kelo. There were notable outliers in the NMDS plot, especially in the fallen kelo and fungal-infected living trees, probably a result of sample heterogeneity as the exact age, time of death, and the length of time fallen kelo were in contact with the ground are not known. Also, chemical changes associated with the fire might contribute to this variability, especially in the fallen kelo group as discussed later.

Decaying heartwood from fallen kelo and fungal-infected living trees contained similar amounts of total phenolics and major compounds such as total stilbenes, PS and PSM, but at statistically lower concentrations than the healthy heartwood from living trees. The PS concentrations in living-infected trees was lower than in living trees, but not statistically lower. The other tree groups with less decayed heartwoods contained similar quantities of these compounds as the live trees. Karppanen et al. (2008) measured rapid concentration declines of both PS and PSM with increasing mass loss of Scots pine heartwood decayed by the brown-rot *Coniophora puteana*. They concluded this fungus was capable of eliminating these two compounds, even though they have been identified as playing a major part in heartwood decay resistance (e.g., Venäläinen et al. 2004; Harju et al. 2009). This interpretation fits our observations here as well. Other notable chemical differences include VA-d with higher concentrations in living trees with *Phellinus pini* sporocarps, in which the

heartwood had mostly disintegrated followed by fallen kelo and standing kelo types (Fig. 2e). This suggests a possible correlation between advancing fungal infestation and VA-d production. Then there is Fa-d which was similar in all the groups except in living trees with no visible fungal attack, where it was strikingly higher (Fig. 2g). The role of all these compounds in fungal decay or resistance can be explored further. .

Phenolic compounds have been previously linked to defence mechanisms of plants against natural enemies, such as fungal pathogens (Hammerschmidt 2003). However, no consistent conclusive evidence has come up confirming the relationship between phenolics and the resistance of trees to pathogens from previous literature (Witzell and Martín 2008). Our findings give rise to few possibilities: Instead of directly attributing to the tree resistance, these phenolics especially the minor ones may act as precursors for other defensive compounds or may act as a group rather than as individual compounds. Since marked differences between living trees and standing kelos were not observed, it is also possible that the heartwood phenolics could be complemented by other defensive metabolites such as terpenoids or defensive proteins. This could help us to understand the 'possible [ecological] uniqueness of kelos' (Niemelä et al. 2002). However both these theories require further investigation.

The segregation of the trees with and without fire scars in the ordination plot was rather clear, although this aspect was not directly included in the original sampling plan. Thus, due to the scarcity of data, we do not have conclusive evidence to prove that fire plays a role in the formation of kelo chemistry. However, it was interesting to note that the trees in the 'living' and 'living with *Phellinus pini* fruiting bodies' type with fire scars tend to resemble those kelos that have charred trunks. The grouping of the samples in the NMDS plot was highly

correlated to axis 2 ($R^2$=.902), denoting the environmental variable 'presence/absence of fire scar' and indicating the role of fire in the phenolic chemistry and its transition in the different substrates. Furthermore, most of the phenolics (except for higher concentration of neolignans and more or less similar concentration of stilbenes, other than PS and PSM) were comparatively less abundant in the fire scarred trees (Fig. 5b). Earlier, Harju et al. (2008) showed that the concentration of resin acids, stilbenes and lignans increased when injury was induced to the xylem in Scots pine. Quite contrary to this, our results indicate that overall phenolic concentration was lower in fire scarred individuals.

The absence of sufficient differences in phenolic chemistry between living trees and standing kelos contradicts our initial hypothesis that the apparent uniqueness of kelos can be explained by secondary phenolics. Our results indicated fungal infestation and fire having notable effect on the phenolic composition of the deadwood, in accordance with our second hypothesis. It is highly likely that this also affects the decomposition rate of the woody tissues. . However, our results suggested that the chemical distinctiveness among the living tree groups and the kelo groups were limited and it became evident only in stages of tree death and advanced decay. This supports our third hypothesis that the woody phenolic composition changes when a tree advances from one growth or decay stage to another. Despite our new findings, there are still wide gaps in our understanding of extractive formation and their dependence on environmental factors, tree growth (especially radial growth), and silvicultural practices such as thinning. A better assessment of these relationships would provide more information in regard to the manipulation of artificial kelo formation and wood quality, and also for understanding the decomposition dynamics of the trees in forest ecosystems.

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**Table 1.** Features of the different Scots pine tree types (Fig. 1.) used in the study

| Tree type | Specific properties and identifiers |
|---|---|
| Living* | i. Healthy standing Scots pine trees with no visible fungal sporocarps |
| | ii. 100 % intact bark |
| | iii. Diameter at breast height (dbh): 40–50 cm |
| Living with fungi* | i. At least one dead or living *Phellinus pini* sporocarp attached to the tree trunk |
| | ii. 100 % intact bark |
| | iii. Dbh: 30–35 cm |
| | iv. Intact sapwood but partially disintegrated heartwood core |
| Fallen non-kelo | i. Trees uprooted by wind |
| | ii. 80–100% bark; wood still hard |
| | iii. Diameter at about 140 cm from the base: 30–35 cm |
| | iv. Mostly decay class: I-II (Vanderwel et al. 2006); Mostly intact heartwood core |
| Partial kelo* | i. Standing, but still partly living Scots pine trees |
| | ii. Less than 20 % bark, grayish trunk and fewer branches; on the way to be formed into kelo |
| | iii. Dbh: 40–48 cm |
| Standing kelo* | i. Standing long-ago died, decorticated Scots pines with silvery grey trunk |
| | ii. Either whole crown absent from the standing trunk or the presence of just a few thick branches |
| | iii. Sapwood mostly worn-off leaving eroded heartwood |
| | iv. Dbh: 40–45 cm |
| Fallen kelo* | i. Long-ago fallen kelo |
| | ii. Mostly decay class II-III (Knife penetrates to wood when pushed); sapwood completely eroded leaving only disintegrating heartwood core |
| | iii. Diameter at about 140 cm from the base: 45-55 cm |

*Two individuals among the 'living' tree group; one in 'living with fungi'; two in 'partial kelo'; four in 'standing kelo' and five in 'fallen kelo' had fire scars on trunk

**Table 2**. Mass accuracy (or error term) of the phenolic compounds identified through UPLC-DAD-MS.

| Stilbene proposed | Identified compound | $M_{measured}$ (Monoisotopic mass) | $M_{calculated}$ (Accurate mass) | Mass accuracy (in ppm) |
|---|---|---|---|---|
| $stilbene_1$ | PS | 211.0769 | 212.0758 | 5.07 |
| $stilbene_2$ | PSM | 225.0936 | 226.0994 | 9.33 |
| $stilbene_4$ | Resveratrol | 227.0719 | 228.0786 | 5.23 |
| $stilbene_5$ | Methyl Resveratrol | 241.0877 | 242.0943 | 5.57 |
| $stilbene_6$ | Methyl Piceatannol | 257.0823 | 258.0892 | 3.78 |

**Table 3.** Concentration (Means ± 1 S.E.) of the individual and total phenolics (mg/g dry wood weight) present in the heartwood of different Scots pine tree types F- and p-values are based on one-way ANOVA. Asterisks (*) denote *P*-values: *$p < 0.05$; **$p < 0.01$.

| Phenolic Groups | Serial Nr. | Phenolics | Living (n=8) | Living with fungi (n=7) | Fallen non-kelo (n=5) | Partial kelo (n=5) | Standing kelo (n=8) | Fallen kelo (n=5) | F-value | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Stilbene | 1 | $ST_1$ | 2.79 ± .73 | 0.98 ± .28 | 3.13 ± .42 | 3.14 ± 1.84 | 1.44 ± .30 | 0.34 ± .16 | 2.399 | .059 |
| | 2 | $ST_2$ | 7.04 ± 1.45 | 1.72 ± .47 | 7.43 ± 1.58 | 8.85 ± 3.22 | 5.32 ± .80 | 1.50 ± .67 | 4.056 | .006* |
| | 3 | $ST_3$ | 0 | 0.49 ± .26 | 0 | 0 | 0 | 0.01 ± .01 | 3.167 | .020* |
| | 4 | $ST_4$ | 0.02 ± .01 | 0.07 ± .03 | 0.001 ± .00 | 0.01 ± .00 | 0.004 ± .00 | 0.01 ± .01 | 2.555 | .047* |
| | 5 | $ST_5$ | 0 | 0.09 ± .02 | 0 | 0 | 0 | 0 | | |
| | 6 | $ST_6$ | 0 | 0 | 0 | 0 | 0 | 0.05 ± .09 | | |
| | 7 | $ST_7$ | 0.03 ± .03 | 0.12 ± .04 | 0.03 ± .01 | 0.12 ± .05 | 0.06 ± .01 | 0.04 ± .02 | 2.017 | .103 |
| | 8 | $ST_8$ | 0 | 0.02 ± .01 | 0 | 0 | 0 | 0 | | |
| | 9 | $ST_9$ | 0.18 ± .03 | 0.04 ± .01 | 0.14 ± .03 | 0.23 ± .07 | 0.14 ± .03 | 0.05 ± .02 | 4.525 | .003* |
| | 10 | ST-d | 0.33 ± .19 | 0.32 ± .29 | 0.06 ± .02 | 0.40 ± .29 | 1.71 ± 1.00 | 0.84 ± .30 | 1.259 | .306 |
| | | Total | 10.38 ± 2.04 | 3.85 ± .98 | 10.79 ± 2.05 | 12.75 ± 5.43 | 8.67 ±.77 | 2.83 ± .82 | 3.172 | .028* |
| Neolignan | 11 | $NL_1$ | 0.44 ± .17 | 0.09 ± .09 | 0.07 ± .07 | 0.66 ± .35 | 0.80 ± .43 | 0.18 ± .14 | 1.263 | .304 |
| | 12 | $NL_2$ | 0 | 0 | 0 | 0.04 ± .02 | 0 | .031 ± .02 | 4.034 | .006* |
| | 13 | $NL_3$ | 0 | 0 | 0.01± .01 | 0.08 ± .05 | 0.04 ± .02 | 0.01 ± .01 | 5.749 | .001** |
| | 14 | $NL_4$ | 0.07 ± .01 | 0.02 ± .010 | 0.03 ± .01 | 0.05 ± .01 | 0.04 ± .01 | 0.02 ± .01 | 5.501 | .001* |
| | 15 | $NL_5$ | 0.20 ± .05 | 0.01 ± .01 | 0.03 ± .02 | 0.10 ±.03 | 0.08 ± .02 | 0.13 ± .05 | 4.890 | .002* |
| | 16 | $NL_6$ | 0.05 ± .01 | 0 | 0.04 ± .01 | 0.05 ± .02 | 0.04 ± .01 | 0.02 ± .01 | 3.563 | .011* |
| | 17 | $NL_7$ | 0.49 ± .11 | 0.20 ± .06 | 0.52 ± .13 | 0.74 ± .13 | 0.53 ± .10 | 0.15 ± .05 | 4.366 | .004* |
| | | Total | 1.27 ± .20 | 0.31 ± .11 | 0.70 ± .17 | 1.65 ± .55 | 1.45 ± .37 | 0.55 ± .13 | 3.549 | .012* |
| Lignan | 18 | $L_1$ | 0 | 0 | 0.04 ± .03 | 0 | 0 | 0.20 ± .17 | 1.815 | .138 |
| | 19 | $L_2$ | 0.06 ± .00 | 0 | 0.04 ± .01 | 0.01 ± .01 | 0.02 ± .01 | 0.03 ± .02 | 4.879 | .002* |
| | 20 | $L_3$ | 0.05 ± .00 | 0 | 0.05 ± .01 | 0.05 ± .02 | 0.04 ± .01 | 0.03 ± .03 | 2.239 | .074 |
| | 21 | $L_4$ | 0 | 0.02 ± 0.01 | 0.01 ± .01 | 0 | 0 | 0 | 1.548 | .203 |
| | 22 | $L_5$ | 0 | 0.01 ± .01 | 0.02 ± .01 | 0.03 ± .03 | 0 | 0.03 ± .03 | 1.094 | .383 |
| | 23 | $L_6$ | 0 | 0.09 ± .04 | 0.11 ± .03 | 0 | 0.004 ± .004 | 0 | 5.480 | .001** |
| | 24 | $L_7$ | 0 | 0 | 0.01 ± .01 | 0.02 ± .02 | 0 | 0 | 2.046 | .099 |
| | 25 | $L_8$ | 0.05 ± .01 | 0.02 ± .01 | 0.05 ± .01 | 0.11 ± .04 | 0.11 ± .06 | .01 ± .01 | 1.615 | .184 |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 26 | $L_9$ | 0.10 ±.10 | 0.05 ± .05 | 0 | 0.30 ± .11 | 0.13 ± .09 | 0 | 1.446 | .235 |
| | 27 | $L_{10}$ | 0.35 ± .25 | 0 | 0.06 ± .02 | 0.08 ± .01 | 0.08 ± .02 | 0.04 ± .01 | 1.056 | .403 |
| | 28 | $L_{11}$ | 1.23 ± 1.20 | 0 | 0 | 0.04 ± .01 | 0.34 ± 22 | 0 | 0.670 | .649 |
| | 29 | $L_{12}$ | 0.04 ± .01 | 0 | 0 | 0.03 ± .00 | 0.03 ± .01 | 0.02 ± .01 | 5.435 | .001** |
| | | Total | 1.88 ± 1.56 | 0.18 ± .06 | 0.39 ± .06 | 0.67 ± .14 | 0.74 ± .32 | 0.34 ± .20 | 0.621 | .685 |
| Vanillic acid derivatives | 30 | VA-$d_1$ | 0 | 0.02 ± .01 | 0.003 ± .00 | 0.003 ± .00 | 0.01 ± .00 | 0.02 ± .01 | 5.213 | .001** |
| | 31 | VA-$d_2$ | 0.003 ± .001 | 0.01 ± .00 | 0.01 ± .00 | 0.02 ± .01 | 0.02 ± .01 | 0.01 ± .00 | 1.670 | .170 |
| | 32 | VA-$d_3$ | 0 | 0.10 ± .06 | 0 | 0 | 0 | 0 | | |
| | 33 | VA-$d_4$ | 0 | 0.01 ± .00 | 0 | 0 | 0 | 0 | | |
| | 34 | VA-$d_5$ | 0.04 ± .01 | 0.24 ± .08 | 0.01 ± .00 | 0.02 ± .01 | 0.06 ± .03 | 0.14 ± .06 | 4.608 | .003* |
| | | Total | 0.04± .01 | 0.38 ± .09 | 0.03 ± .00 | 0.04 ± .01 | 0.09 ± .03 | 0.17 ± .06 | 8.308 | .001** |
| Cinnamic acid derivative | 35 | CA-d | 0.01 ± .00 | 0.02 ± .01 | 0.02 ±.00 | 0.01 ± .00 | 0.01 ± .00 | 0.01 ± .00 | 0.523 | 0.757 |
| Ferulic acid derivatives | 36 | FA-$d_1$ | 0.01 ± .00 | 0 | 0.01 ± .01 | 0.003 ± .01 | 0 | 0 | 2.416 | .058 |
| | 37 | FA-$d_2$ | 0 | 0.004 ± .00 | 0.04 ± .01 | 0.04 ± .02 | 0.03 ± .01 | 0 | 5.580 | .001** |
| | 38 | FA-$d_3$ | 0.06 ± .02 | 0.01 ± .00 | 0.01 ± .00 | 0.02 ± .01 | 0.02 ± .00 | 0.01 ± .00 | 4.083 | .006* |
| | 39 | FA-$d_4$ | 0.16 ± .03 | 0 | 0 | 0 | 0 | 0 | | |
| | | Total | 0.23 ± .05 | 0.01 ± .01 | 0.05 ± .01 | 0.06 ± .02 | 0.05 ± .01 | 0.01 ± .00 | 12.125 | .000** |
| Naringenin derivatives | 40 | NG-$d_1$ | 0 | 0 | 0.02 ± .01 | 0.03 ± .01 | 0.04 ± .01 | 0.02 ± .01 | 5.860 | .001** |
| | 41 | NG-$d_2$ | 0 | 0.07 ± .03 | 0 | 0 | 0 | 0 | | |
| | 42 | NG-$d_3$ | 0.17 ± .02 | 0 | 0.10 ± .04 | 0 | 0.16 ± .02 | 0 | 17.334 | .000** |
| | | Total | 0.17 ± .02 | 0.07 ± .03 | 0.11 ± .04 | 0.03 ± .01 | 0.20 ± .02 | 0.02 ± .01 | 7.518 | .000** |
| Eriodictyol derivatives | 43 | ER-$d_1$ | 0.03 ± .01 | 0 | 0.08 ± .04 | 0 | 0.03 ± .00 | 0 | 5.208 | .001** |
| | 44 | ER-$d_2$ | 0.02 ± .01 | 0 | 0 | 0 | 0.03 ± .01 | 0 | 11.120 | .000** |
| | 45 | ER-$d_3$ | 0.05 ± .01 | 0 | 0 | 0 | 0.07 ± .01 | 0 | 23.029 | .000** |
| | 46 | ER-$d_4$ | 0.12 ± .04 | 0.07 ± .02 | 0 | 0 | 0.10 ± .02 | 0 | 5.112 | .001* |
| | 47 | ER-$d_5$ | 0.13 ±.02 | 0.05 ± .03 | 0.12 ± .03 | 0.15 ± .05 | 0.12 ± .02 | 0.04 ± .02 | 2.354 | .063 |
| | 48 | ER-$d_6$ | 0.04 ± .01 | 0 | 0.02 ± .01 | 0 | 0.06 ± .01 | 0 | 17.277 | .000** |
| | | Total | 0.38 ± .06 | 0.12 ± .04 | 0.22 ± .03 | 0.15 ± 0.05 | 0.41 ± .04 | 0.04 ± .02 | 10.416 | .000** |
| Total phenolics concentration | | | 14.35 ± 3.26 | 4.94 ± 1.13 | 12.30 ± 2.24 | 16.04 ± 7.71 | 11.61 ± 1.04 | 3.98 ± 1.06 | 2.794 | .034* |

Note: Compounds present in only one group of trees were not analyzed statistically.

25

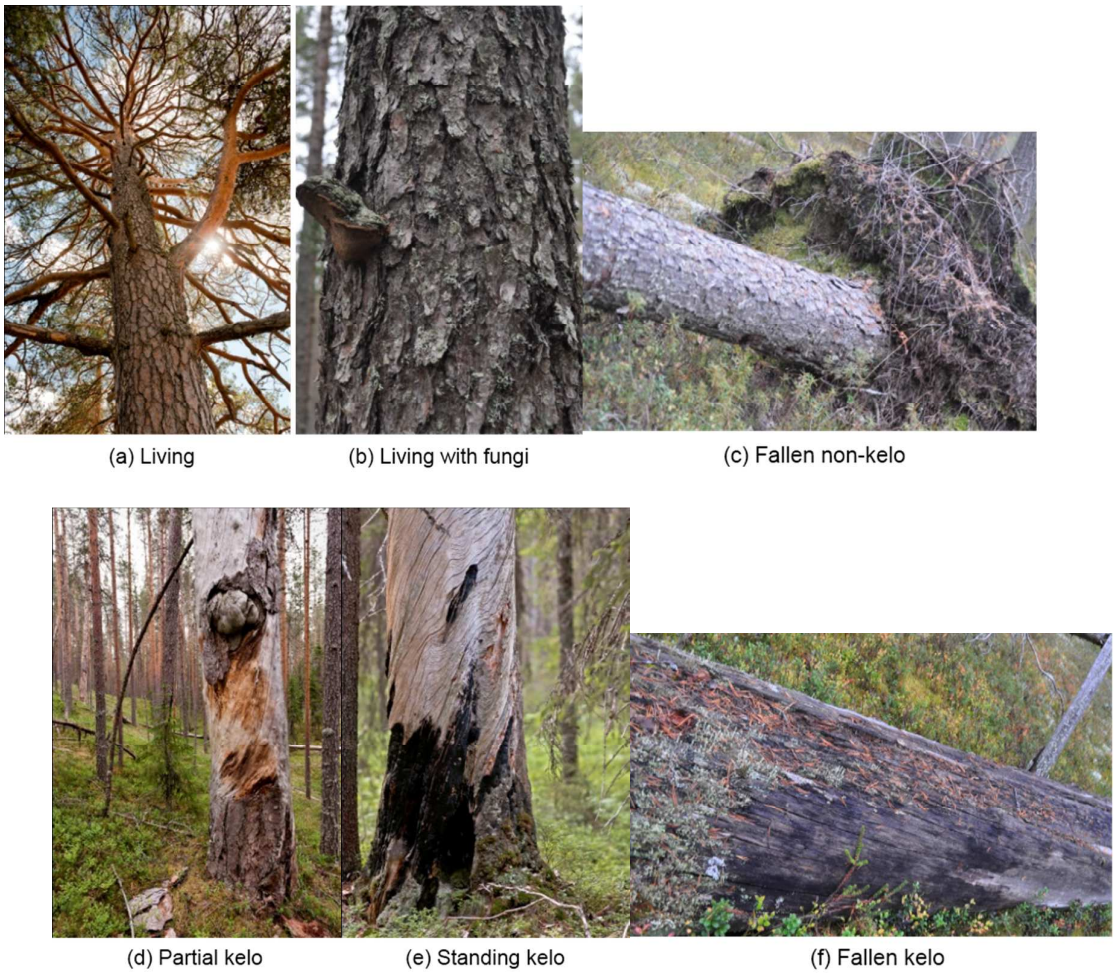


**Fig. 1.** The Scots pine tree types included in the study (for characteristic features, see Table 1).

26

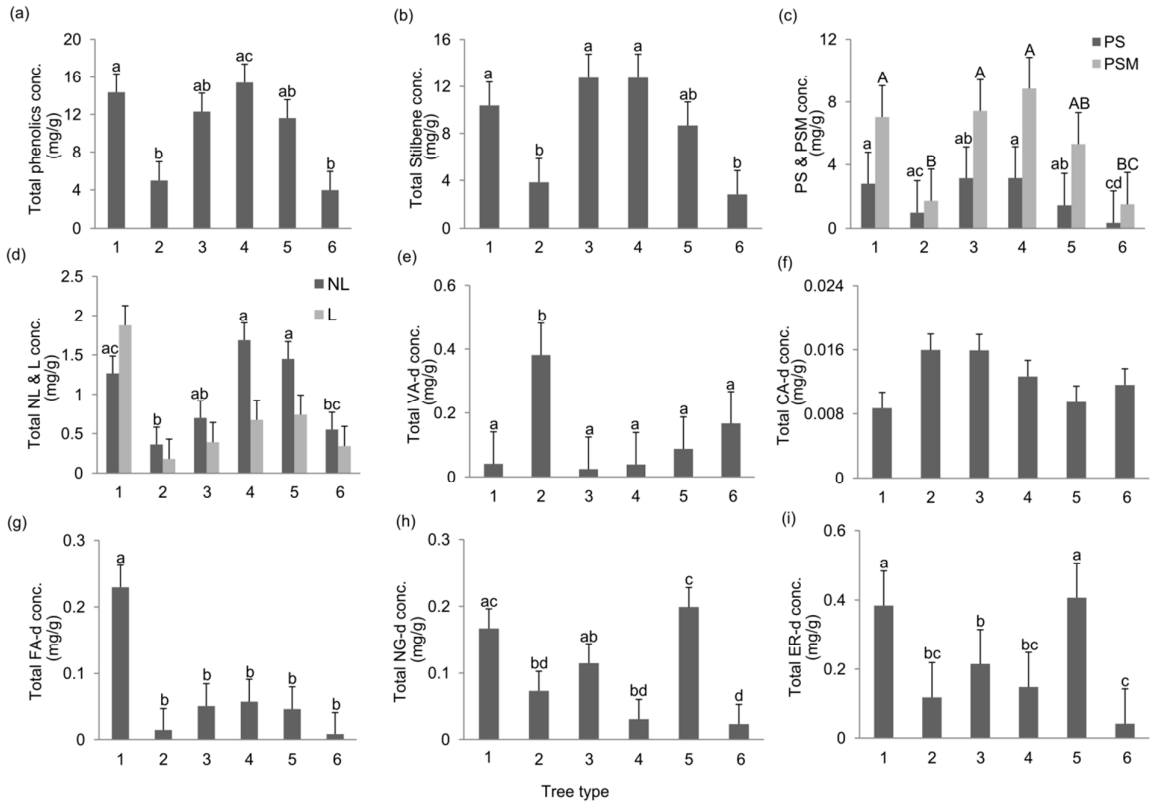


**Fig. 2.** Phenolic concentrations (mg/g, mean + 1 SE) in the heartwood from the six Scots pine tree types: 1) living, 2) living with fungi, 3) fallen non-kelo, 4) partial kelo, 5) standing kelo and 6) fallen kelo. Figure includes (a) total phenolics (b) stilbenes (c) pinosylvin and pinosylvin monomethyl ether (d) neolignans and lignans (e) vanillic acid derivatives, (f) cinnamic acid derivative, (g) ferulic acid derivatives, (h) naringenin derivatives and (i) Eriodictyol derivatives. The post-hoc differences using LSD test are indicated by the letters 'a-d'and 'A-C' Within each panel, bars topped by the same/shared letter are not significantly different according to LSD poc-hoc test at p = 0.05

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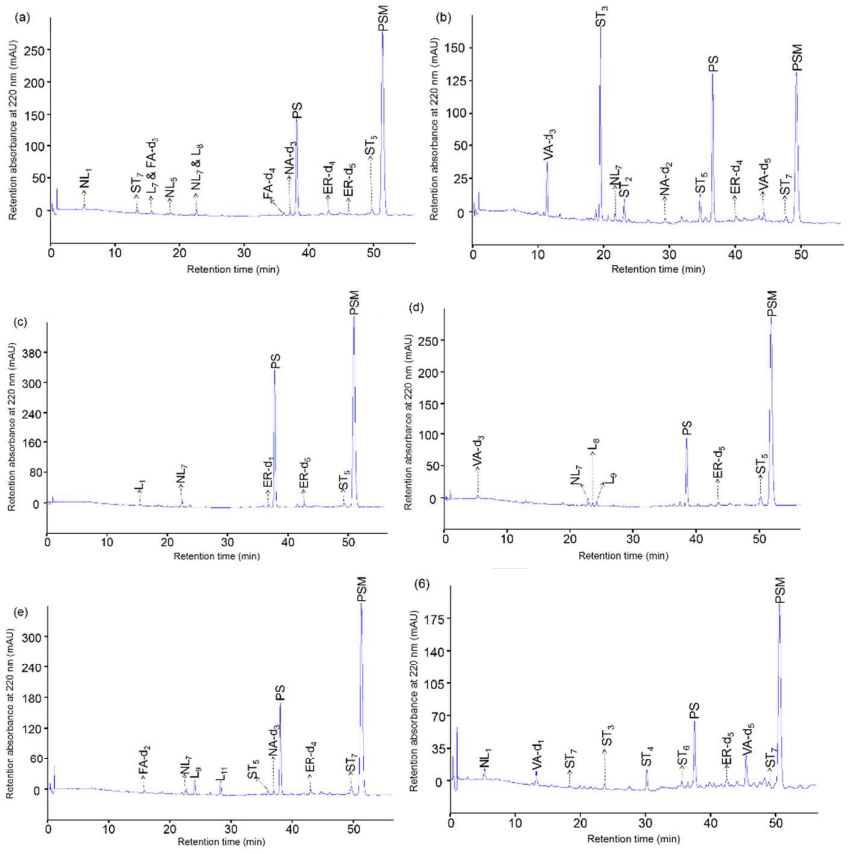


**Fig. 3.** Spectral chromatograms of one tree sample per each tree type showing the most typical spectral signature characteristics for each tree group (a) living, (b) living with fungi, (c) fallen non-kelo, (d) partial kelo, (e) standing kelo and (f) fallen kelo.

28

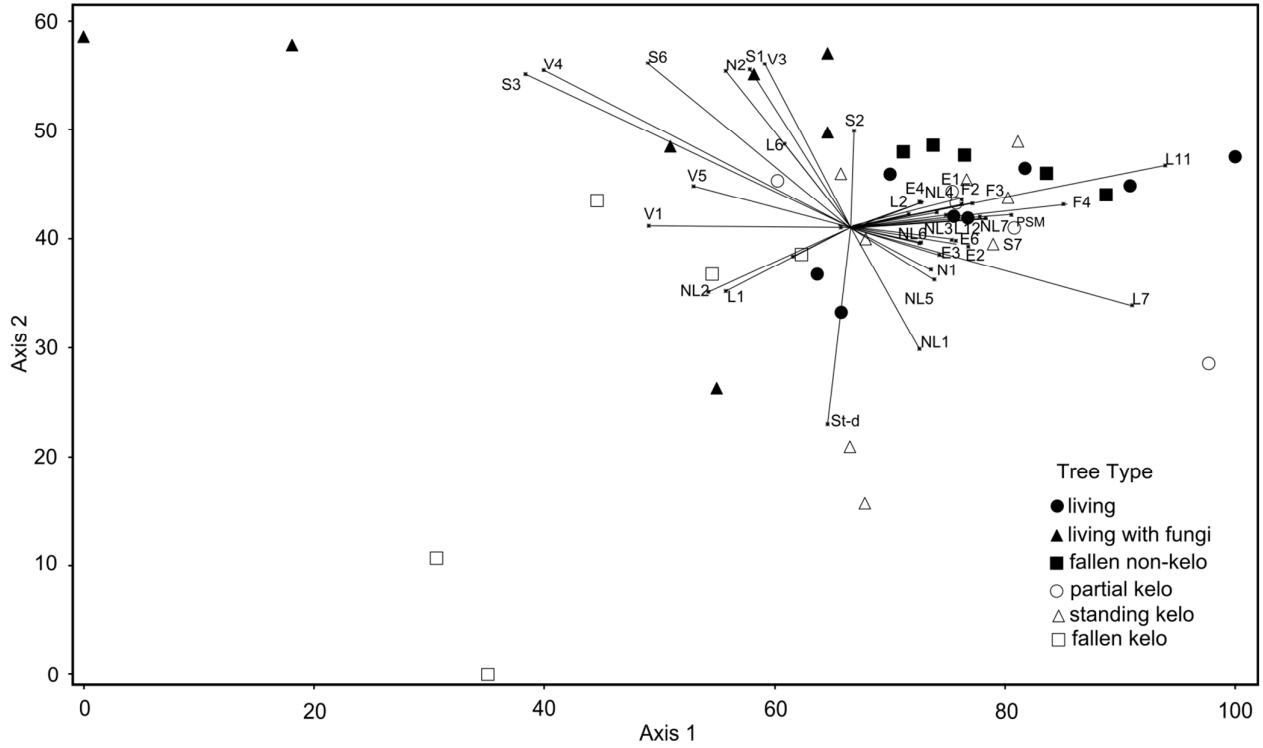


**Fig. 4**. Non-Metric multi-Dimensional Scaling (NMDS) ordination of the tree types, based on their phenolic composition. The compounds (NL, L, PS, PSM and St-d retaining their abbreviated forms and 'V' for vanillic acid derivative, 'E' for eriodictyol derivative and 'F' for ferulic acid derivatives; these were found to be significant earlier in the ANOVA analysis) are displayed based on their relative role in the alignment of the different tree type individuals. (The $R^2$ value of axis 1 was 0.689 and that of axis 2 was 0.902).

29

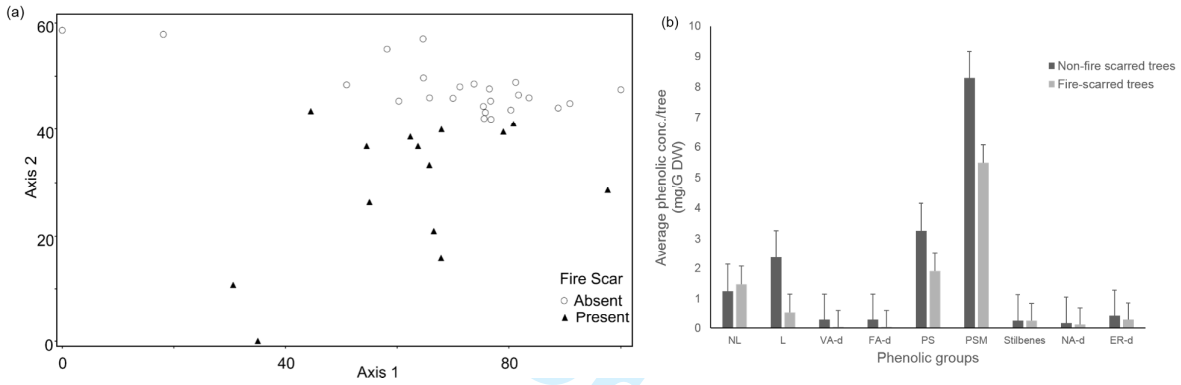


**Fig. 5**. (a) Non-Metric multi-Dimensional Scaling (NMDS) ordination of the tree samples, separating samples with and without visible fire scars on their trunk. (b) Phenolic concentrations (mg/g DW, mean + 1 SE) in the heartwood between trees with and without fire scars.