# FTIR-ATR spectra as fingerprints of forest floor composition

## Uso de los espectros FTIR-ATR como huellas dactilares de la composición del suelo forestal

M. Getino-Álvarez[1,2,*], R. San-Martin[2,3] & M.B. Turrión[1,2]

[1] Dpto. Ciencias Agroforestales, ETSIIAA Palencia, Universidad de Valladolid, España
[2] Instituto Universitario de Investigación en Gestión Forestal Sostenible, UVa-INIA. ETSIIAA Palencia, España
[3] Dpto. Estadística e Investigación Operativa, ETSIIAA Palencia, Universidad de Valladolid, España
(*E-mail: marina.getino@uva.es)

**A B S T R A C T**

It's known that soil has a great potential for C sequestration, but it is also important to point out the contribution of forest ecosystems to the mitigation of global warming. Mixed forest stands has arouse great relevance in forestry research. However, it is necessary to increase the knowledge about quantity and quality of its soil organic matter (SOM), and species mixture effect on its decomposition dinamics. Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) is a powerful analytical tool for molecular characterization of plant materials. Its use with litter samples may simplify its processing and characterization. This study analizes forest floor of mixed and pure stands of Scots pine (*Pinus sylvestris*) and Sessile oak (*Quercus* sp.) with FTIR-ATR, to see if this analysis allows us to: separate litter samples by species, decomposition state, and determine if decomposition dynamics are affected by the stand type. A principal component analysis and Linear mixed models were applied. Results showed that FTIR-ATR analysis is a useful tool in the study of litter composition. This fast and economic analysis allows to differentiate *(p<0.01)* between species, as well as litter humification state. Finally, results showed that litter decomposition dynamics are affected by the type of mass *(p<0.01)*.

**Keywords:** FTIR-ATR, SOM, mixed forests, litter decomposition dinamics

**R E S U M E N**

Los ecosistemas forestales tienen un gran potencial para la captura de C, contribuyendo de a mitigar el calentamiento global. En las últimas décadas, los bosques mixtos han despertado gran interés en la investigación forestal. Sin embargo, es necesario incrementar el conocimiento sobre la cantidad y calidad de su MOS, y el efecto de la mezcla de especies sobre la dinámica de descomposición de la hojarasca. La reflectancia total atenuada por espectroscopia infrarroja transformada de Fourier (FTIR-ATR) es una poderosa herramienta analítica para la caracterización molecular de materiales vegetales. Su uso con muestras de hojarasca puede simplificar su procesamiento. Este estudio analiza la hojarasca de rodales puros y mixtos de pino silvestre (*Pinus sylvestris*) y roble albar (*Quercus* sp.) con FTIR-ATR, para comprobar si permite: separar las muestras por especie, por estado de descomposición, y determinar si la dinámica de descomposición se ve afectada por el tipo de rodal. Los resultados mostraron que el análisis FTIR-ATR es una herramienta útil en el estudio de la composición de la hojarasca. Este rápido y económico análisis permite diferenciar *(p<0,01)* entre especies, así como el estado de humificación de la hojarasca. Finalmente, los resultados mostraron que la dinámica de descomposición de la hojarasca si se ve afectada por el tipo de masa *(p<0.01)*.

**Palabras clave:** FTIR-ATR, MOS, bosques mixtos, descomposición de la hojarasca

## INTRODUCTION

Within the framework of a global climate crisis soils constitute a valuable tool to combat climate change due to its great potential for C sequestration. Soils store two-thirds of the total carbon in terrestrial ecosystems and contain more C than vegetation and the atmosphere combined. Therefore, changes in the loss-gain dynamics of soil organic carbon (SOC) can lead to large changes in the carbon balance at a regional scale (Schlesinger & Andrews, 2000).

Apart from the role of soils, it is also important to point out the contribution of forest ecosystems to the mitigation of global warming. The main inputs of C in forest ecosystems came from litter and the decomposition of plant roots. Depending on tree species composition the leaf litter production will be different and so quantity and quality (chemical composition) of the soil organic matter (SOM) inputs (Román Dobarco *et al.*, 2021). Thus, it is reasonable to believe that C accumulation and decomposition dynamics may vary depending on tree species composition.

In the last decades, mixed stands have arouse great relevance in forestry research, due to the evidence that mixed forests can supply numerous ecosystem services more efficiently (Bravo-Oviedo *et al.*, 2014; Pretzsch *et al.*, 2015). Depending on the management applied, forests may increase considerably their capacity to accumulate SOC, or become sources of $CO_2$ that returns to the atmosphere. The SOC changes in forest floor (organic horizons) are highly relevant for C sequestration, since these horizons are more sensitive to changes in plant cover and management (Stockmann *et al.*, 2013). In order to propose management alternatives considering the soil as a carbon sink, it is necessary to increase the knowledge about quantity and quality of its SOM, and the effect of species mixture on its decomposition dinamics, an aspect that has been poorly studied.

Traditional processing of litter samples, involving separating them into three groups according to their decomposition state (fresh, fragmented and humified), requires a tedious manual sorting and large amounts of time. Furthermore, when working in mixed forests, it is also necessary to separate litterfall by species, which makes laboratory work even more difficult.

Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) is a powerful analytical tool for identifying organic and inorganic compounds (Lammers, 2008). It allows molecular characterization of plant materials in a fast, sensitive, economical way and only requires a small amount of sample (Román Dobarco *et al.*, 2021).

The present study aims to determine if the FTIR-ATR spectra are fingerprints of the information existing in the litter. Specifically, knowing if the FTIR-ATR spectra allow us to: (1) separate litter samples according to species, (2) separate litter samples according to decomposition status, and (3) determine if decomposition dynamics are affected by the stand type (pure or mixed).

## MATERIALS AND METHODS

### *Study site*

For this study, two triplets (six forest plots) of mixed and pure stands of Scots pine (*Pinus sylvestris*) and Sessile oak (*Quercus* sp.) were selected. These belong to a European network of triplets (COST Action FP 1206 EuMiXFOR). Each triplet with pure and mixed plots (one per stand type; pine, oak, mixed) of the species under study (*Pinus sylvestris* and *Quercus* sp.). Plots within a triplet present similar site conditions (soil, climate, relief), age, density and management.

The triplets studied were located in Aguilar de Campoo (Castilla y León), North Spain (Figure 1). It has an oceanic-continental climate (Cfb) according to Köppen classification, with a mean annual temperature of 9.4 °C and annual precipitation of 782 mm. Plots are located at an altitude of 1193–1239 meters above sea level. Soils are Cambisols developed on sandstones, conglomerates and lutites, with sandy-loam texture and acid pH (4.1 - 5.2).

Stand characteristics of the studied plots are the following: stand age between 50 years, basal area between 42 and 66.2 m² ha⁻¹, stand density between 846.9 and 1426.6 trees ha⁻¹, dominant height between 20.3 and 21.7 m. No silvicultural
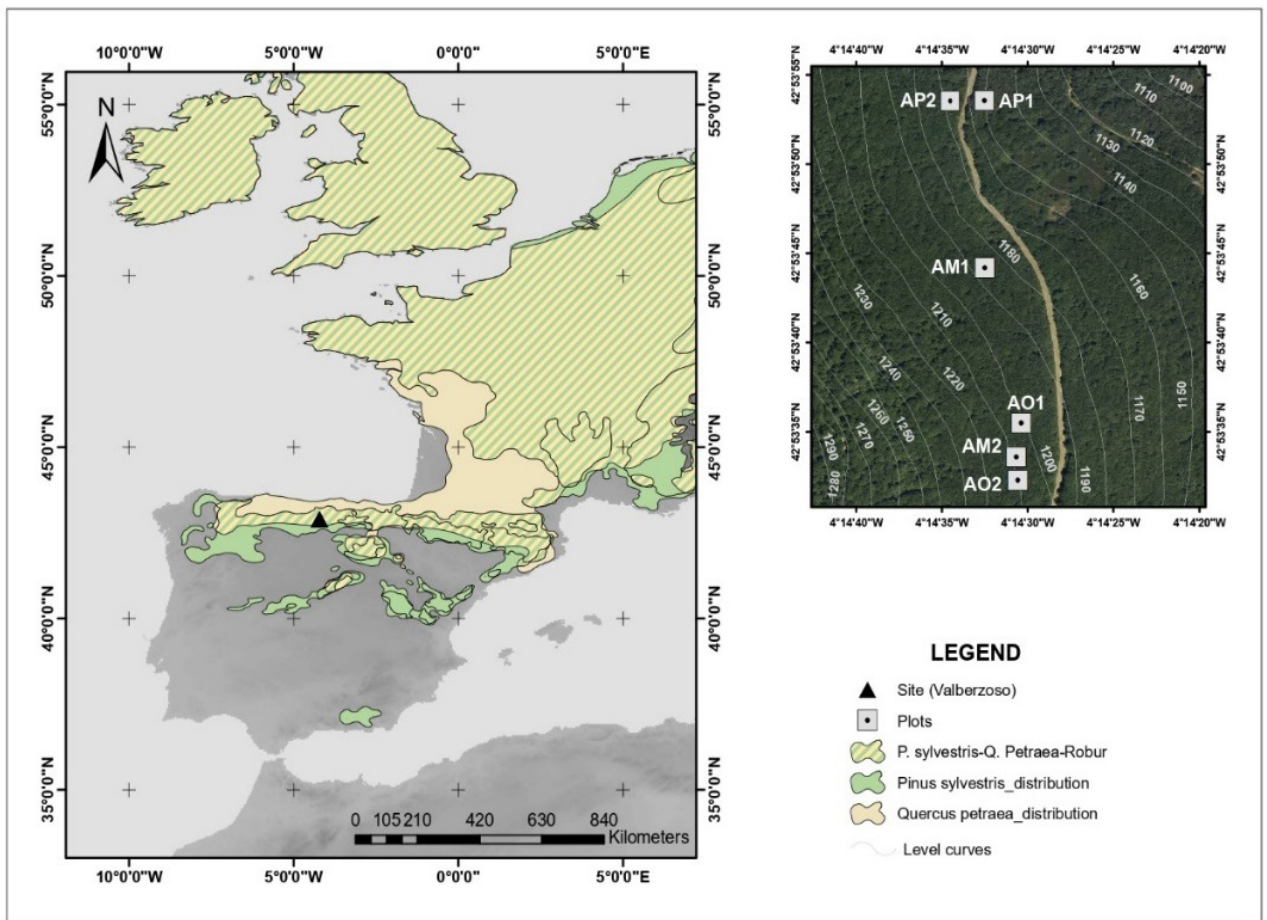
**Figure 1 -** Study site location, Aguilar de Campoo, Palencia (Spain).

intervention has been done in the stands in the last ten years.

### Experimental design and litter sampling

In order to cover spatial variability, 26 circular subplots of 5 m radius were established within the previously described plots avoiding overlapping between subplots. In general, four subplots were established in pure stands and five in mixed stands. To provide a representative forest floor (organic horizon) sample of the area, samples were taken at three points 1 m apart from the center of each subplot, and then mixed creating one composite sample per subplot. Forest floor (FF) was collected using a 25x25 cm wooden frame as a reference area.

### Laboratory analysis

Forest floor samples were separated into three fractions according to decomposition state as in López-Marcos *et al.* (2018): almost undecomposed litter or fresh fraction (FsL), partially decomposed litter or fragmented fraction (FgL) and mostly decomposed organic matter or humified fraction (HmL).

Litterfall fractions were dried, finely grounded and scanned with FTIR-ATR (Nicolet iS50 FT-IR-ATR). The reflectance of the sample was recorded at a resolution of 4 cm$^{-1}$ and 64 scans per spectrum. All spectra were visualized, processed and analysed using OMNIC software.

A principal component analysis (PCA) was performed on 17 variables; FTIR-ATR absorbance bands (A1031, A1154, A1204, A1233, A1259, A1317, A1370, A1418, A1445, A1512, A1539, A1559, A1614, A1711, A1729, A2838, A2919). In order to check significant differences between cases Linear mixed models were applied.

## RESULTS AND DISCUSSION

Principal component axes one and two accounted for 78.47% of the data set variance (Figure 2). The PC2 explains 21.16% of the variance and seems to be related to a higher absorbance of aromatic compounds such as lignin (A1500-A1539). The remaining absorbance bands are correlated with PC1, which explains the 57.31% of the data set variance.

Regarding the first objective, this analysis does allow us to differentiate between litter types (species) and decomposition states of this litter. We can differentiate the litter of the two species throught PC2: oak litter samples showed positive values and significantly different (*p<0.01*) from pine litter,

which showed negative values. Pine litter spectra have a higher absorbance in the band from 1500 to 1559 cm$^{-1}$ indicating a higher lignin content. Second objective was also reached, being able to separate litter samples according to decomposition state. The fresh litter spectra presented positive values for both principal components (upper-right quadrant). The fragmented litter was distributed in the intermediate zone, with positive values for PC1 and negative values for PC2, except for the oak (upper-left quadrant). All humified fractions showed negative values for PC1 (left quadrant). Finally, significant differences (*p<0.01*) were observed in litter fractions (FsL, FgL and HmL) depending on the stand type. So, litter decomposition dynamics are affected by the type of mass (pure or mixed).

## CONCLUSIONS

The FTIR-ATR analysis seems to be a useful tool in the study of litter composition in mixed stands. This fast and economic analysis allows to differentiate between species, as well as between the different stages of decomposition, and see the effect of species mixture on litter decomposition dynamics.
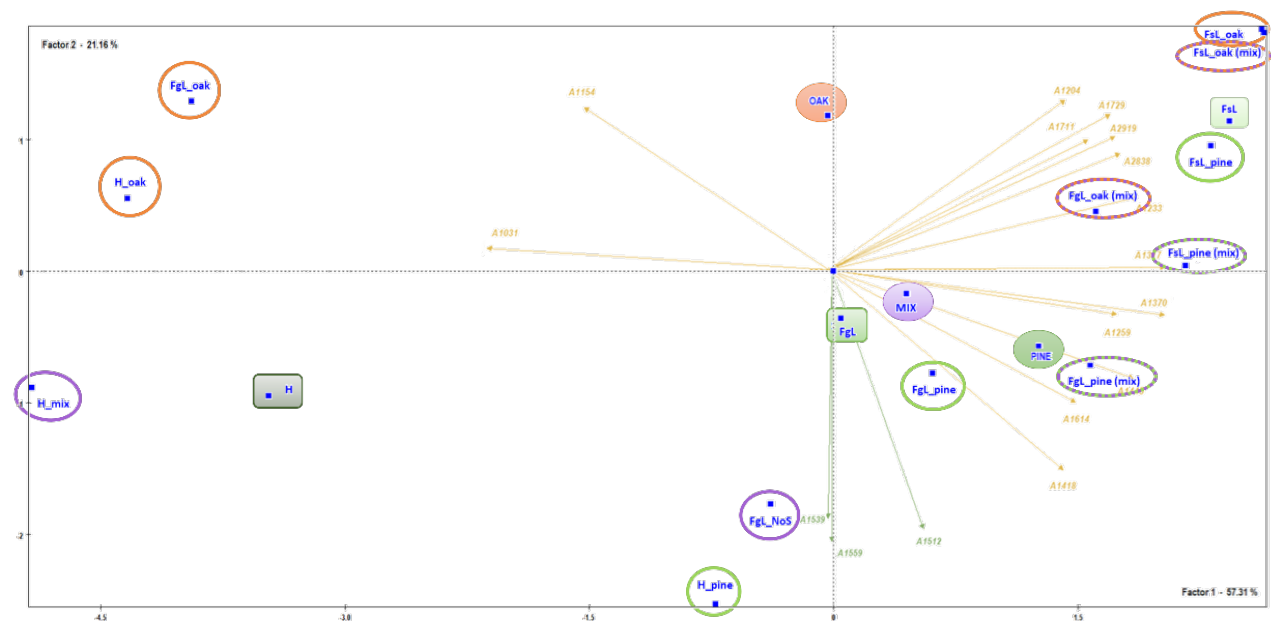


**Figure 2 -** Plot of variables and centroids of cases on the two-first PCA axes, gathering 78.47% of dataset variation. Green circles: pine litter; orange circles: oak litter; purple circles: litter in mixed stands.  Squares: litter decomposition state.

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