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Characterization of woody roots located in dikes by near-infrared spectroscopy and chemometrics

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36 diameter class. Commonly used approaches are buried litterbags and trench plots or more rarely
37 tethered roots and buried pots (Silver & Miya, 2001). Chemical analysis generally target C, N, lignin
38 and polyphenol concentrations which are commonly used to characterize and predict decomposition
39 stages (Creed & al., 2004; Goebel & al., 2011). So some authors as Aulen & al., (2012) used Near-
40 Infrared spectroscopy (NIRS) to assess the chemical characteristics of root samples. Indeed, NIRS is
41 commonly used to identify and predict wood physical and chemical properties (Poke & Raymond,
42 2006). NIRS presents a strong potential, comparable in efficiency to traditional chemiometric
43 methods but with considerable time saving (Malkavaara & Raimo, 1998; Kelley & al., 2004; Jones &
44 al., 2006). Indeed, near-infrared spectroscopy is faster, requires less sample preparation and may be
45 non-destructive (Marten & al., 1989). Spectroscopic techniques allows investigation at molecular
46 scale for complex samples, information on sample chemical composition and physical properties and
47 possible interactions. NIRS was already successfully used to characterize and discriminate different
48 trees species (Çetinkol & al., 2012).

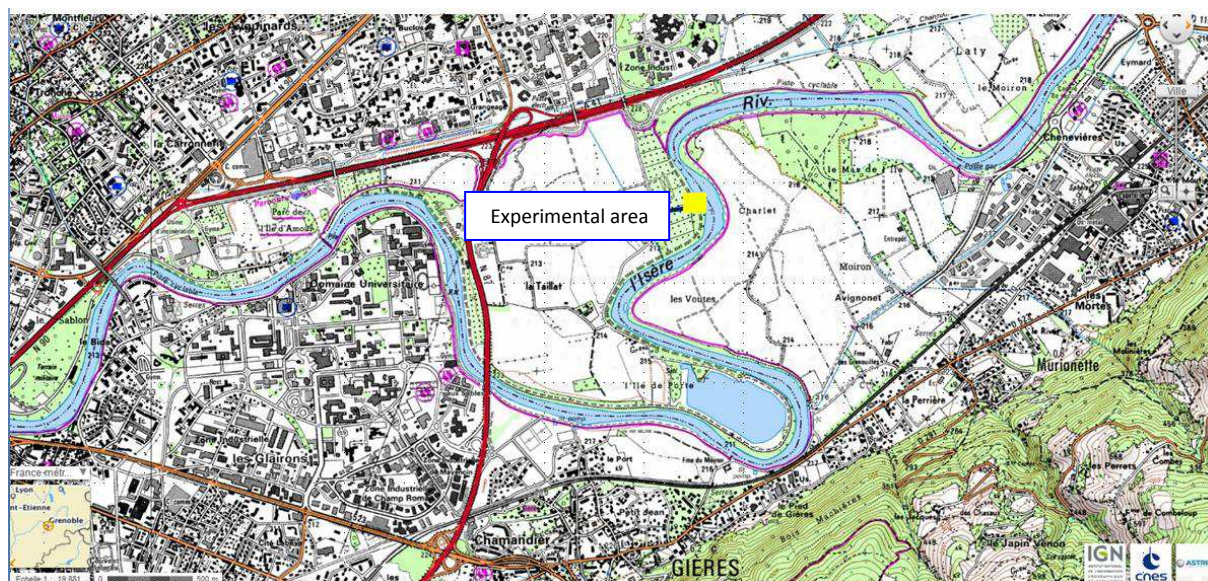
49 **Study goals**

50 This study aims at contributing to the assessment of risk related to the presence of root systems and
51 their decomposition in embankment dikes in order to help designing vegetation management plan.
52 This paper focuses on the characterization of NIRS response of decaying roots from tree species
53 frequently growing on embankment dikes. Root decomposition causing chemical and physical
54 changes in the wood, it is important to characterize the variability of these changes as a function of
55 decomposition time, tree species and root diameter.

56 **Material and methods**

57 **Experimental device**

58 In order to study root decomposition of various species in homogeneous conditions, an experimental
59 device was laid out in 2008 on a dike of Isere River (Fig.1) (Caroline Zanetti & al., 2008).



60
61

Fig. 1. Location of experimental area.

62 This old dike was built in the 19th century from non-compacted heterogeneous materials (a mixture
63 of sand, gravel and silt in various proportions) and heightened between 1950 and 1970. Isère dikes
64 are narrow (ridge 3-meter wide) with steep slopes covered up to recently by a dense forest stand
65 (Pinhas, 2005). Therefore they are vulnerable to internal erosion caused by the presence and
66 potential decay of large woody roots (Zanetti & al., 2009). Oak (*Quercus robur*), Ash (*Fraxinus
67 excelsior*), Poplar (*Populus alba*) and Black Locust (*Robinia pseudoacacia*), the most frequent tree
68 species on French dikes, were selected for the experiment. After tree logging, stumps were carefully
69 extracted from dike slopes or ridge in order to preserve root systems for further investigation. Root
70 samples (length = 20 cm, diameters 3, 5, 8 and 10 cm) were immediately cut from these stumps, laid
71 out in stainless steel baskets and buried at 50 cm depth in the embankment (Fig. 2). The experiment
72 has been designed for a 10-year long follow up of root decomposition, roots sample collection being
73 scheduled every two or three years according to their evolution.

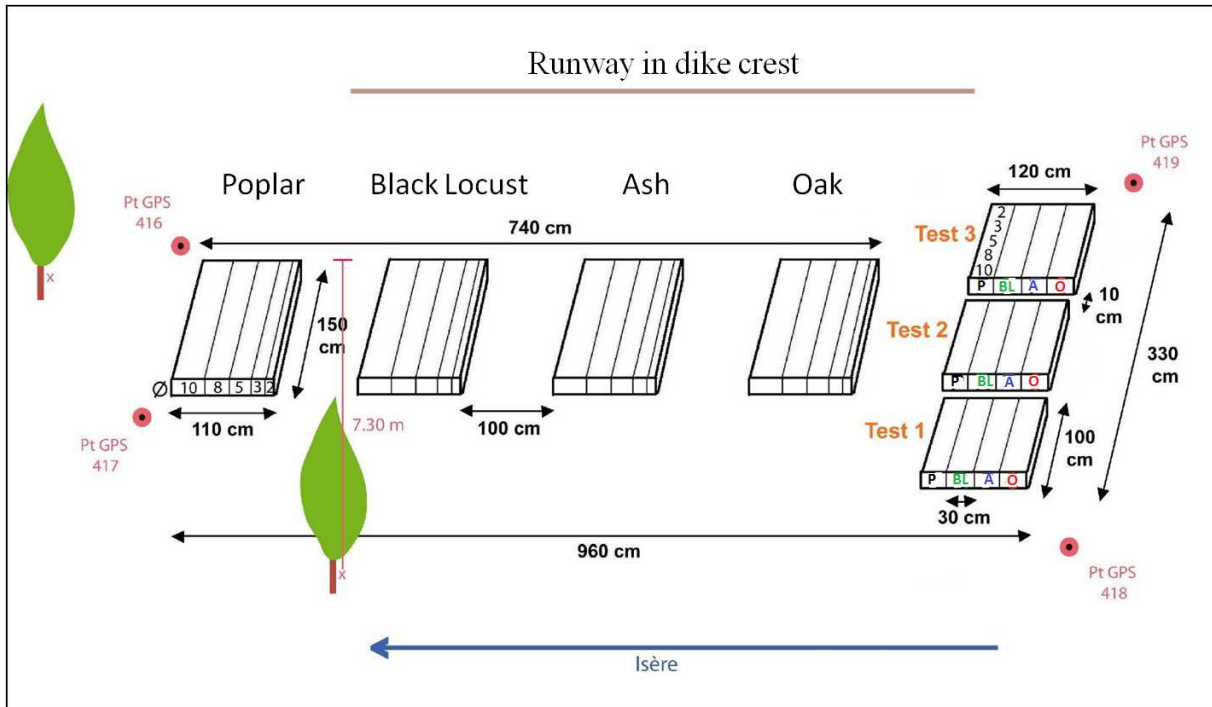


Fig. 2. Root decomposition experimental device.

74
75

76 In 2008, the first samples were analyzed in order to measure wood initial characteristics for each
77 species and root diameter. To date, we successively analysed root samples at three steps of the
78 decaying process: T0 (initial wood - 2008), T2 (2 years of decomposition - 2010) and T4 (4 years of
79 decomposition - 2012).

80 Near-Infrared spectroscopy

81 We used a FT-NIR spectrophotometer, Thermo-Nicolet Antaris equipped with an interaction sphere
82 (spectral range: $10000-4000\text{cm}^{-1}$, 50 scans, 4 cm^{-1}). All samples were dried at room temperature
83 (25°C) and then cut in slices with the same saw to obtain the required quantity of sawdust. For each
84 species and diameter, three samples were used in order to obtain three spectra which were
85 subsequently averaged (Fig.3). For chemometric study, all spectra were pretreated with a first
86 derivative.

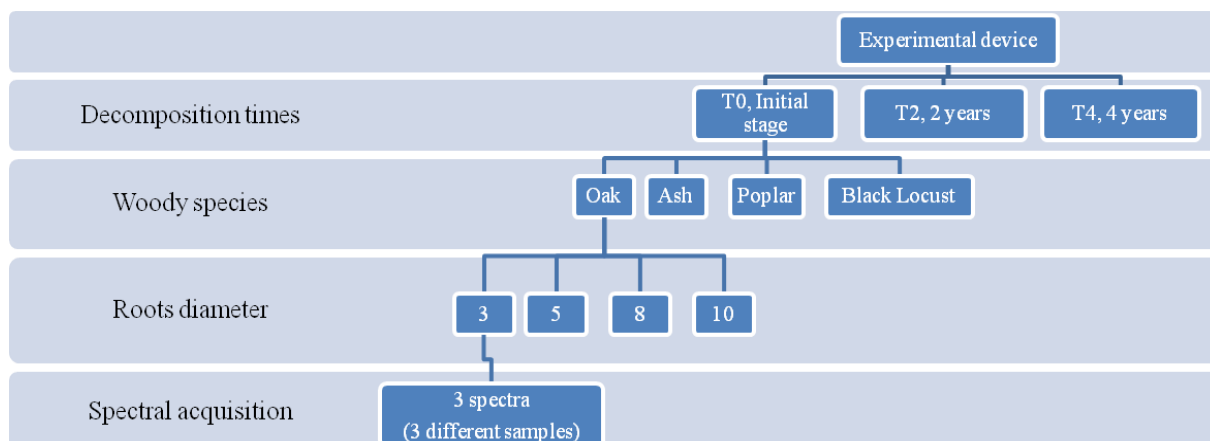


Fig. 3. Presentation of the different classes of samples.

87
88

89 The sample set is therefore composed of: 3 (three samples per diameter)* 4 (four diameter classes)*
90 4 (four tree species)* 3 (three decomposition time) = 144 samples.

91 **Data processing: chemometric methods**

92 PCA was used as a first step for exploratory data analysis to detect groups and investigate data
93 structure.

94 PLS (Haaland & Thomas, 1988) was initially designed for quantitative analysis, but it is now also used
95 for pattern recognition. This supervised analysis is based on the relation between spectral intensity
96 and sample characteristics (Martens & Næs, 1989).

97 The prediction of decomposition times was performed with a PLS 1 model. Samples were split into
98 two sets: 96 samples for calibration and validation (cross-validation) and the 48 remaining ones to
99 test the predictive model. For each decomposition time, a PLS2-DA model was computed to predict
100 tree species and root diameter simultaneously. The sample was then assigned to one class when the
101 value was above a specific prediction threshold (Roussel & al., 2003).

102 To predict tree species, the output variable was transformed with a binary code: 1 for the samples
103 belonging to the class and 0 to those not belonging to the class. Samples with predicted values
104 between 0.5 and 1.5 were identified as belonging to the class and those with values out of these
105 limits as not belonging to the class. Samples were again split into two sets: 32 to perform calibration
106 and validation (cross-validation) and 16 to test model predictions. Models accuracy was assessed
107 with performance indices: R² (calibration), SEC (standard error of calibration), bias (calibration),
108 number of latent variables, R² (prediction), SEP (standard error of prediction) and bias (prediction).
109 For discriminant analysis (PLS-DA), the confusion matrices allowed accessing the percentages of
110 correct classification.

111 This chemometric study was conducted with the Unscrambler software version 9.8 from CAMO
112 (Oslo, Norway).

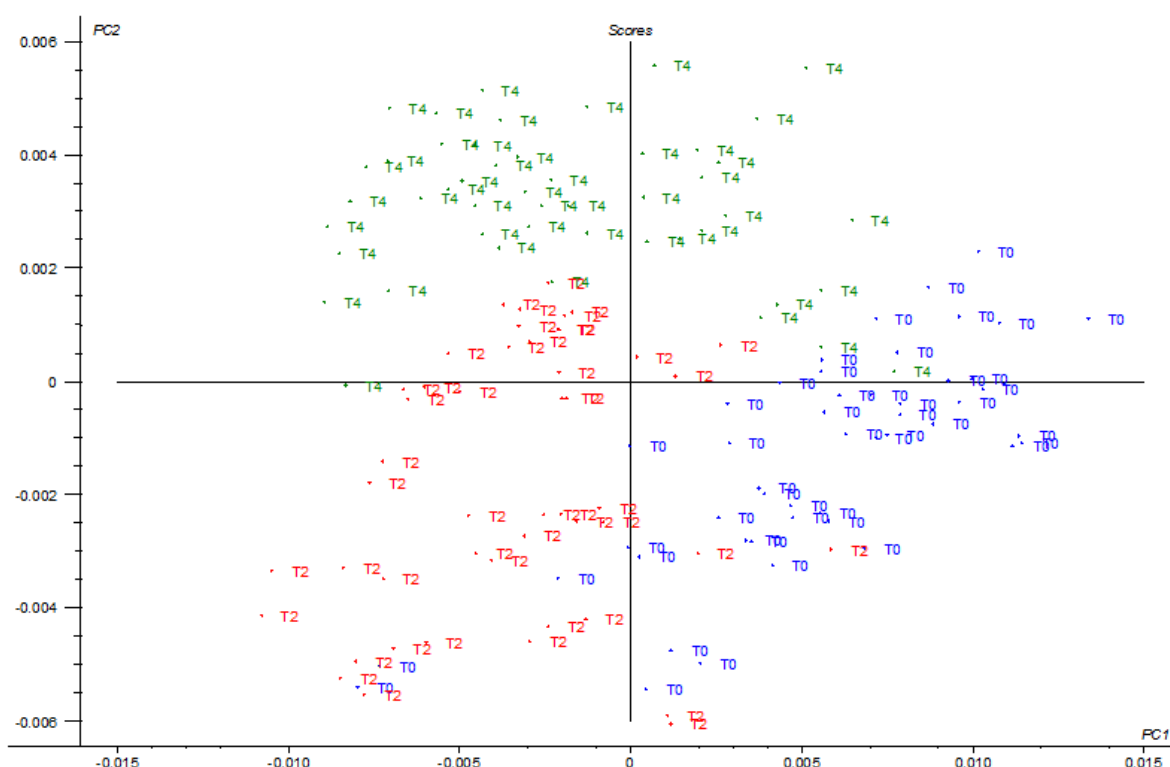
113 **Results and discussion**

114 **Exploratory analysis (Principal Component Analysis - PCA)**

115 The PCA on NIRS spectra highlighted groups, subgroups, the dispersion within groups and spectral
 116 ranges of interest.

117 ***Separation of root decomposition times;***

118 In the first plane of the PCA with all root samples (n = 144, Fig. 4) the three decomposition times (T0,
 119 T2, and T4) clearly gathered in 3 distinct groups and therefore constitute the first criterion to sort
 120 samples. The observed dispersion within groups was linked to the other sources of variability,
 121 particularly tree species and root diameter (Fig. 5). For all samples, the general trend with ageing
 122 seemed to be similar, regardless of tree species and diameter classes.

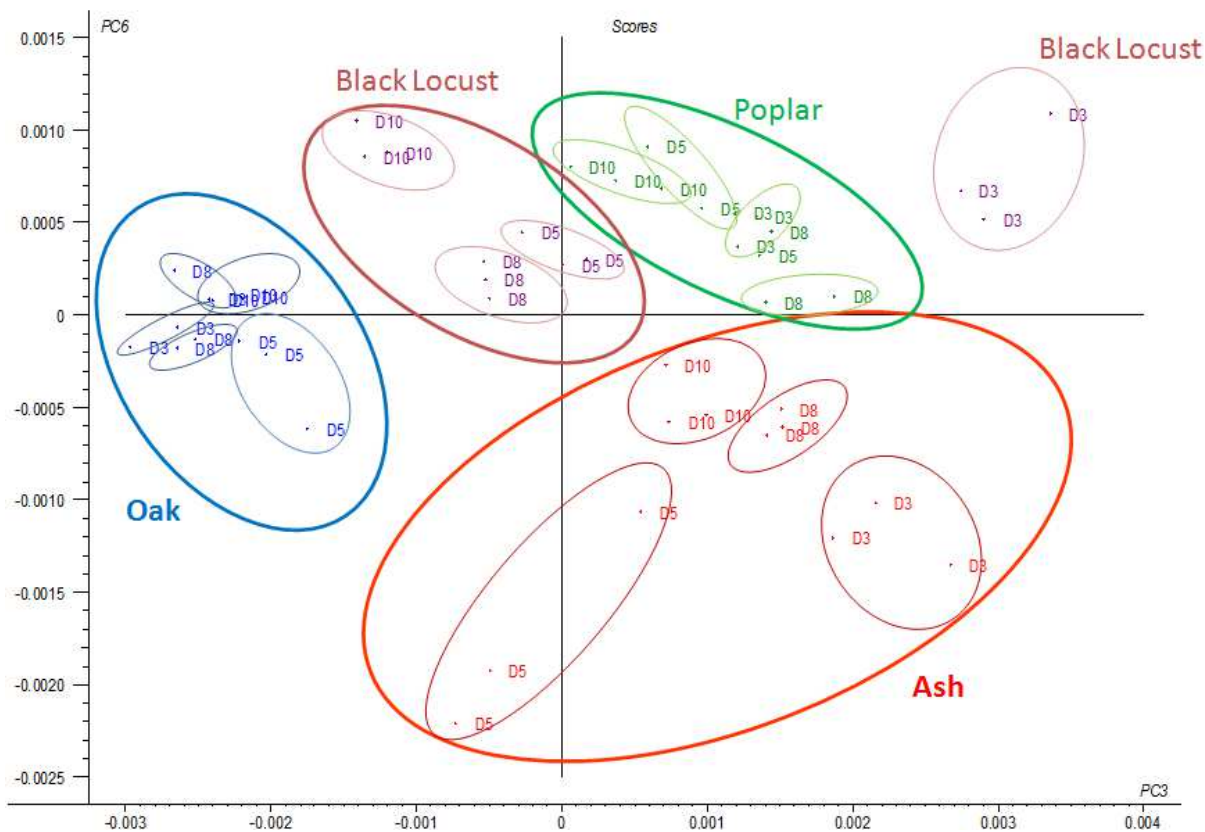


123
 124 **Fig. 4. Plan 1/2 of the PCA conducted on all samples (T0 = initial state, T2 = 2 year and T4 = 4 years, n = 144, explained**
 125 **variance = 78%).**

126 ***Tree species and root diameter***

127 The three PCA performed separately for each decomposition time (T0, T2 or T4) highlighted groups
 128 corresponding to the four tree species: oak, ash, poplar and black locust and subgroups related to
 129 the four diameter classes. There are significant differences in chemical composition and physical
 130 properties between tree species and for each species between the different diameters at all
 131 decomposition times. However, the more samples are ageing (after four years of decomposition), the
 132 less the variability between diameters. On figure 5 showing the PCA performed at decomposition

133 times T0, a good separation between tree species, as well as between diameter classes within each
 134 species was obtained in the plane of main components 3 and 6. Species constitutes the second
 135 discrimination criterion (after decomposition time) except for black locust which diameter class 3
 136 stands apart from the rest of the species area. Root diameter is the third criterion.



137
 138 **Fig. 5. Plan 3/6 of the PCA conducted on woody species and diameter classes at decomposition times T0 (Blue = Oak, red**
 139 **= Ash, green= poplar, purple = Black Locust, D3=diameter 3, D5=diameter 5, D8=diameter 8, D10=diameter 10, n = 48,**
 140 **explained variance = 9%).**

141 **Predictive analysis (Partial Least Square Regression – PLS regression)**

142 The goal of this analysis was to calibrate the model in order to further assess its ability to
 143 differentiate unknown root samples belonging to different species.

144 **Prediction of root decomposition times (PLS1)**

145 The PLS-1 model allowed predicting the decomposition time with a good correlation (R^2), an error of
 146 prediction (SEP) and a low bias (Table 1).

147

148 **Table 1. Statistics for the predictive model of decomposition times (PLS1).**

Decomposition Times	NIR
	T0, T2 and T4
Factor number	6
R ² (Calibration)	0,981
SEC	0,314
Bias (Calibration)	- 7,45.10 ⁻⁸
R ² (Prediction)	0,952
SEP	0,506
Bias (Prediction)	0,016

R²: coefficient correlation, SEC: Standard Error of Calibration, SEP: Standard Error of Prediction

149

150 In the prediction model, the three following margins were considered for acceptance:

151 - For T0, -1 to 1; for T2, 1 to 3; for T4, 3 to 5.

152 Two T0 samples were predicted as T2; one T2 sample was predicted as T0; and one T4 sample was
153 predicted as T2. Finally, only 4 samples among 48 were misclassified, less than 10%.

154 This shows the good accuracy of the model for predicting decomposition time of unknown samples.

155 ***Prediction of woody species and the diameter of roots (PLS2-DA)***

156 The PLS2-DA models allowed predicting tree species and diameter for each of the three
157 decomposition times with a good correlation (R²), few errors of prediction (SEP) and low bias (Table
158 2). Concerning tree species, the model led to an accurate prediction (Table 3).

159

160 When comparing statistics of the three models, prediction for oak and black locust became slightly
161 less accurate for T2 and T4 than for T0. For poplar, we did not observe significant variations between
162 the different decomposition times. Finally, we observed a decrease in prediction accuracy of ash
163 between T2 and T4.

164

165 The confusion matrix for tree species showed that only three samples were misclassified. The
166 prediction considered successively each species compared to all other ones ("other"). No
167 classification error was found for T0. For T2, one "other" (ash) sample was predicted as an oak and
168 one poplar sample was classified as "other". For T4, one oak sample of was classified as "other".

169 These good results showed that even after 4 years of decomposition and a degraded physical
170 structure, species still display significantly different chemical composition and physical
171 characteristics.

172

173 **Tableau 2. Statistics for the predictive models (PLS2-DA) of roots diameter and woody species for each decomposition**
 174 **times.**

NIR					
T0					
Predicted variables	Diameter	Oak	Ash	Poplar	Black Locust
Factor number	8	8	8	8	8
R ² (Calibration)	0,999	0,974	0,953	0,983	0,951
SEC	0,110	0,099	0,133	0,081	0,136
Bias (Calibration)	- 5,96.10 ⁻⁸	3,26.10 ⁻⁹	- 3,26.10 ⁻⁹	- 3,72.10 ⁻⁹	4,66.10 ⁻⁹
R ² (Prediction)	0,863	0,972	0,858	0,952	0,914
SEP	1,406	0,107	0,246	0,140	0,194
Bias (Prediction)	0,224	0,020	-0,001	-0,008	-0,011
T2					
Predicted variables	Diameter	Oak	Ash	Poplar	Black Locust
Factor number	8	8	8	8	8
R ² (Calibration)	0,997	0,931	0,938	0,965	0,921
SEC	0,208	0,160	0,152	0,116	0,171
Bias (Calibration)	5,21.10 ⁻⁸	5,59.10 ⁻⁹	- 6,98.10 ⁻⁹	- 1,21.10 ⁻⁸	6,05.10 ⁻⁹
R ² (Prediction)	0,810	0,846	0,861	0,928	0,845
SEP	1,652	0,240	0,243	0,178	0,246
Bias	0,436	0,051	0,010	-0,068	0,007
T4					
Predicted variables	Diameter	Oak	Ash	Poplar	Black Locust
Factor number	7	7	7	7	7
R ² (Calibration)	0,997	0,904	0,829	0,974	0,905
SEC	0,200	0,188	0,246	0,100	0,187
Bias (Calibration)	1,34.10 ⁻⁷	1,72.10 ⁻⁸	- 1,72.10 ⁻⁸	- 1,40.10 ⁻⁹	8,85.10 ⁻⁹
R ² (Prediction)	0,787	0,860	0,788	0,955	0,887
SEP	1,885	0,242	0,294	0,134	0,223
Bias (Prediction)	0,011	-0,012	-0,002	-0,001	0,015

R²: coefficient correlation, SEC: Standard Error of Calibration, SEP: Standard Error of Prediction

175

176

177 **Table 3. Confusion matrix for the woody species variables at decomposition times T0, T2 and T4 from the predictive**
 178 **model PLS2-DA regression (Models: First derivative as pre-treatment).**

		Predicted classes - NIR					
		T0		T2		T4	
Prediction	Real classes	Oak	Others	Oak	Others	Oak	Others
Prediction Oak	Oak (n=4)	4	0	4	0	3	1
	Others (n=12)	0	12	1	11	0	12
Prediction Ash	Ash (n=4)	4	0	4	0	4	0
	Others (n=12)	0	12	0	12	0	12
Prediction Poplar	Poplar (n=4)	4	0	3	1	4	0
	Others (n=12)	0	12	0	12	0	12
Prediction Black Locust	Black Locust (n=4)	4	0	4	0	4	0
	Others (n=12)	0	12	0	12	0	12

179

180 Discussion

181 Although tree roots used in this study present much variability, we demonstrated the interest of NIRS
 182 and PLS models to study and predict with a good accuracy a complex set of characteristics including
 183 wood decomposition time, tree species and root diameter. Indeed the total number of samples (144)
 184 was enough for the study of decomposition time but the number of samples for each diameter
 185 within each species was limited. However, the high level of success in sample classification in all
 186 analysis demonstrated that the method was worth being developed and validated on other sites with
 187 the same species and with other species, and more numerous samples.

188 For the first time, we showed that it was possible to discriminate three levels of variability in wood
 189 with a single NIRS analysis, unlike previous studies such as for example Çetinkol & al. (2012) which
 190 focused on only one source of variation, tree species or wood decomposition time. Moreover, there
 191 is scant literature on large tree root decomposition, none of these papers including NIRS. Most
 192 studies using NIRS analyzed the evolution of chemical traits during fine roots decomposition (Aulen &
 193 al., 2012). Tree root evolution for diameters larger than 2 cm is generally assessed by density loss
 194 (Chen & al., 2001; Ludovici & al., 2002). We also used density loss especially with the X-ray
 195 tomography on the same samples (Zanetti, 2010), showing that decomposition time was easy to
 196 assess. But NIRS allowed discriminating samples from their physical and chemical characteristics and
 197 seems to be an innovative approach to the study large tree roots decomposition.

198 Root separation by diameter in NIRS spectra can be explained by the strong correlation between root
 199 diameter and age (Zanetti & al., 2010). During root aging, as for tree trunk, a central zone of
 200 heartwood appears and develops regularly. For diameter class 2, no or very few heartwood was
 201 found in our samples. For larger roots, heartwood proportion increases with diameter up to more
 202 than 50%. Heartwood is formed when wood cells die and are filled with various compounds aiming at
 203 increasing their strength and their resistance to parasites and diseases (fungus, insects, bacteria,
 204 etc.). These chemical and physical changes in cells result in significant variations of NIR spectra.

205

206 **Conclusion**

207 This study showed that the Near-Infrared spectroscopy (NIRS) presents high potential for the study of
208 woody roots growing in earth dikes. The analysis of NIRS spectra by chemometric tools and PLS
209 models allowed discriminating and predicting the decomposition times (0 to 4 years), tree species
210 and root diameter. Compared to traditional methods (density, X-rays tomography, chemical
211 analysis), these models may allow a simpler, faster and reliable assessment at low cost. Provided that
212 it is validated on other species and regions, this method could help river managers to design
213 appropriate maintenance and management plans to ensure the reliability of embankment dikes.

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292