

# Multilevel $^{13}\text{C}$ -late-wood signatures from seasonal-drought-induced stress on *Pinus pinaster* during last 35 years

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## Abstract

*Keywords:*

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<sup>1</sup> **1. Introduction**

<sup>2</sup> **2. Materials and Methods**

<sup>3</sup> **2.1. Study area**

<sup>4</sup> We developed our study in two areas in north and east-center of the Iberian  
<sup>5</sup> Peninsula (Fig. 1). The areas belong to the most vast native provenance region  
<sup>6</sup> of maritime pine (*Pinus pinaster* Ait.), growing on sandy soils and forming  
<sup>7</sup> large continuous populations at moderate densities (average of 500 - 1000 trees  
<sup>8</sup> per hectare). Average altitude for this region ranges from 900 m to 1000 m  
<sup>9</sup> above sea level. Mean annual temperature is about 11 °C and mean annual  
<sup>10</sup> precipitation is approx. 562 mm. Forest ecosystems of the area are associated  
<sup>11</sup> with oaks (*Quercus ilex* L., *Q. faginea* Lam., and *Q. pyrenaica* Willd.), beeches  
<sup>12</sup> (*Fagus silvatica* L.), and other pine species (*Pinus sylvestris* L., *P. nigra* Arn.  
<sup>13</sup> and *P. halepensis* Mill)

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14     2.2. *Core sampling*

15     Dominant trees in ten sites on the maritime pine forests where core sampled  
16     (5 mm diameter) at chest height (1.3 m). The sample trees were located in five  
17     sites on northern study-site edge and other five sites on the center-east edge.  
18     Two dominant trees were sampled by site, and two core samples were extracted  
19     by tree. The core samples were air dried, sanded, and scanned (1000:1600 ppi).  
20     The tree-ring widths in the scanned images were measured with R-package:  
21     *measuRing* ([Lara et al., 2015](#)), and statistically controlled with *dplR* package  
22     ([Bunn, 2010](#)).

23     A master chronology of *P. pinaster* with strong common signal (EPS >  
24     0.95, SNR > 22), developed with core samples of 150 trees on ten sites of  
25     study area ([Bogino and Bravo, 2008](#)), was used to develop statistical control of  
26     the measured rings. The cross-dating process was developed on four common  
27     regions, defined after clustering tree-dimensional coordinates of the sites, with  
28     each of the clusters having sites at most 80 km of closeness (Figure 2).

29     2.3. *climatic data*

30     We processed a high-resolution gridded dataset (0.11° resol.) of monthly  
31     mean temperatures for peninsular Spain: Spain02, ([Herrera et al., 2015](#)) to  
32     compute Standardized Precipitation Indexes (SPI) across Ebro basin (1971 -  
33     2010). Projection of UTM coordinates of the sample plots to coordinate sys-  
34     tem in climate algorithm was developed with R-package *rgdal* ([Bivand et al.,](#)  
35     [2015](#)). The SPIs were modeled from the extracted spatial precipitations with  
36     R-packages: *raster*([Hijmans, 2015](#)) and *spi* ([Neves, 2012](#)).

37     2.4.  $^{13}\text{C}$ -late-wood signatures

38     One core per site was used for isotopic analysis on late wood of the dated  
39     rings. Each latewood portion of rings was carefully separated from earlywood  
40     with a microtome. Only rings formed after 1974 were analysed. Whole wood  
41     was milled, an aliquot of 100 mg was packed in porous bags and used for cellulose  
42     extraction. The samples were washed in 5 percent NaOH solution twice for 2

43 h at 60 °C in order to remove fats, oils, resins and hemicellulose. In a second  
44 step the lignin was removed with NaClO 2.7% After each treatment the samples  
45 were washed with distilled water and then finally dried overnight at 60 °C.

46 The  $\delta^{13}\text{C}$  was determined using an elemental analyser linked to an isotopic  
47 ratio mass spectrometer via a variable open split interface (Max Plank Institute  
48 for Biogeochemistry, Germany). The results of laboratory were presented in the  
49  $\delta$  notation:

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad (1)$$

50 relative to the internationsl VPDB standard for cabon; where  $R_{sample}$  and  
51  $R_{standard}$  is the fractions of  $^{13}\text{C}/^{12}\text{C}$  for the sample and the standard, respec-  
52 tively. The standard deviation for the repeated analysis (commercial cellulose)  
53 was better than 0.1. was better than 0.1 percent. The calibration versus VPDB  
54 was done by measuring IAEA USGS-24(graphite) and IAEA-CH7 (polyethy-  
55 lene).

### 56 2.5. Multilevel detrending

57 We detrended  $\delta^{13}\text{C}$  series and the extracted SPI with the R-package **B10dry**  
58 ([Lara and Bravo, 2015](#); [Lara et al., 2013](#)). Such a package processes multilevel  
59 data frames (MDFs) containing serial records in initial columns, followed by  
60 recorded times (i.e., months, years, relative times, etc.), and ended with factor-  
61 column levels, with factors being ordered from lower levels (usually a core-  
62 sample replicate, or an annual set of monthly meteorological records) to higher  
63 levels in sampling hierarchy (i.e., plots, sites, or other spatial units).

64 The package holds several functions but we implemented only two of them to  
65 develop the detrending process: **modelFrame** and **muleMan**. The former function  
66 was implemented to normalize both:  $\delta^{13}\text{C}$  in late wood of the core samples and  
67 the SPI. This package normalizes the series by fitting linear mixed-effects models  
68 with function **ringLme**, implementing methods in R-package **nlme**.

69 Two kind of model formulas ara available in the package to assist the de-  
70 trending process: 'lmeForm' and 'tdForm', these characters implement functions

71 with same names (see manual of R-package `B10dry`). We used 'tdForm' to nor-  
72 malize the isotopes, and 'lmeForm' to center the SPI. The second function,  
73 `muleMan`, was used to compute the the signatures between normalized  $\delta^{13}\text{C}$  of  
74 *P. pinaster* late-wood and precipitation indexes via Multilevel correlograms:  
75 Mantel correlograms are computed from distance matrices of normalized series,  
76 and permutation tests ([Goslee and Urban, 2007](#)).

77 **3. Results**

78 Intra-annual  $^{13}\text{C}$ -late-wood signatures (1974-2010) were significant during  
79 Junes (Figure 5) and Augests (Figure 6) of last 35 years. The resting simulated  
80 months were no significant and ommited from the analysis.

81    **4. References**

- 82    Bivand, R., Keitt, T., Rowlingson, B., 2015. rgdal: Bindings for the Geospatial  
83    Data Abstraction Library. URL: <https://CRAN.R-project.org/package=rgdal>. r package version 1.1-1.
- 85    Bogino, S.M., Bravo, F., 2008. Growth response of *Pinus pinaster* ait.  
86    to climatic variables in central spanish forests. Ann. For. Sci. 65, 1–13.  
87    doi:[10.1051/forest:2008025](https://doi.org/10.1051/forest:2008025).
- 88    Bunn, A.G., 2010. Statistical and visual crossdating in r using the dplr library.  
89    Dendrochronologia 28, 251 – 258. doi:[10.1016/j.dendro.2009.12.001](https://doi.org/10.1016/j.dendro.2009.12.001).
- 90    Goslee, S.C., Urban, D.L., 2007. The ecodist package for dissimilarity-based  
91    analysis of ecological data. J. Stat. Softw. 22, 1–19.
- 92    Herrera, S., Fernández, J., Gutiérrez, J., 2015. Update of the Spain02 gridded  
93    observational dataset for EURO-CORDEX evaluation: assessing the effect of  
94    the interpolation methodology. International Journal of Climatology .
- 95    Hijmans, R.J., 2015. raster: Geographic data analysis and modeling. URL:  
96    <https://CRAN.R-project.org/package=raster>. r package version 2.4-30.
- 97    Lara, W., Bravo, F., 2015. BI0dry: Multilevel Modeling of Dendroclimatological  
98    Fluctuations. CRAN. URL: <https://cran.r-project.org/web/packages/BI0dry/index.html>.
- 100    Lara, W., Bravo, F., Maguire, D., 2013. Modeling patterns between drought and  
101    tree biomass growth from dendrochronological data: A multilevel approach.  
102    Agric. For. Meteorol. 178-179, 140 – 151. doi:[10.1016/j.agrformet.2013.04.017](https://doi.org/10.1016/j.agrformet.2013.04.017).
- 104    Lara, W., Bravo, F., Sierra, C., 2015. measuRing: An R package to measure  
105    tree-ring widths from scanned images. Dendrochronologia 34, 43 – 50. doi:[10.1016/j.dendro.2015.04.002](https://doi.org/10.1016/j.dendro.2015.04.002).

<sup>107</sup> Neves, J., 2012. spi: Compute SPI index. URL: <https://CRAN.R-project.org/package=spi>.

<sup>108</sup> r package version 1.1.

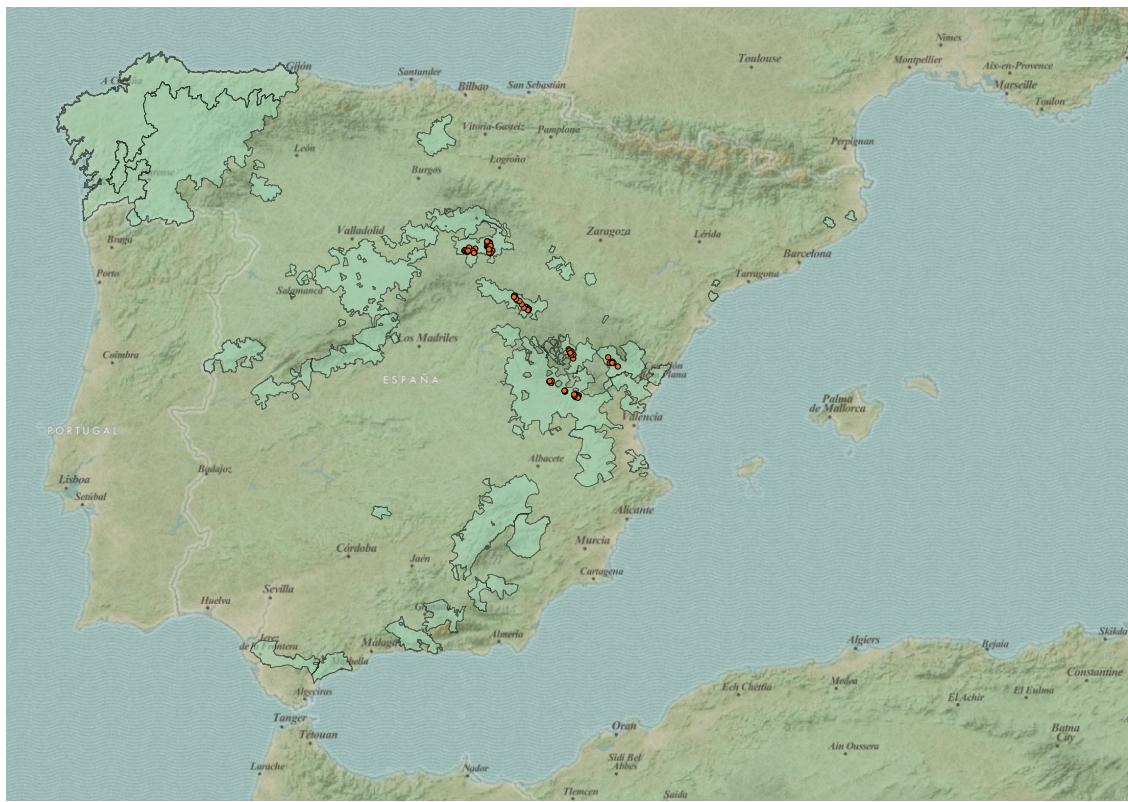


Figure 1: Study site

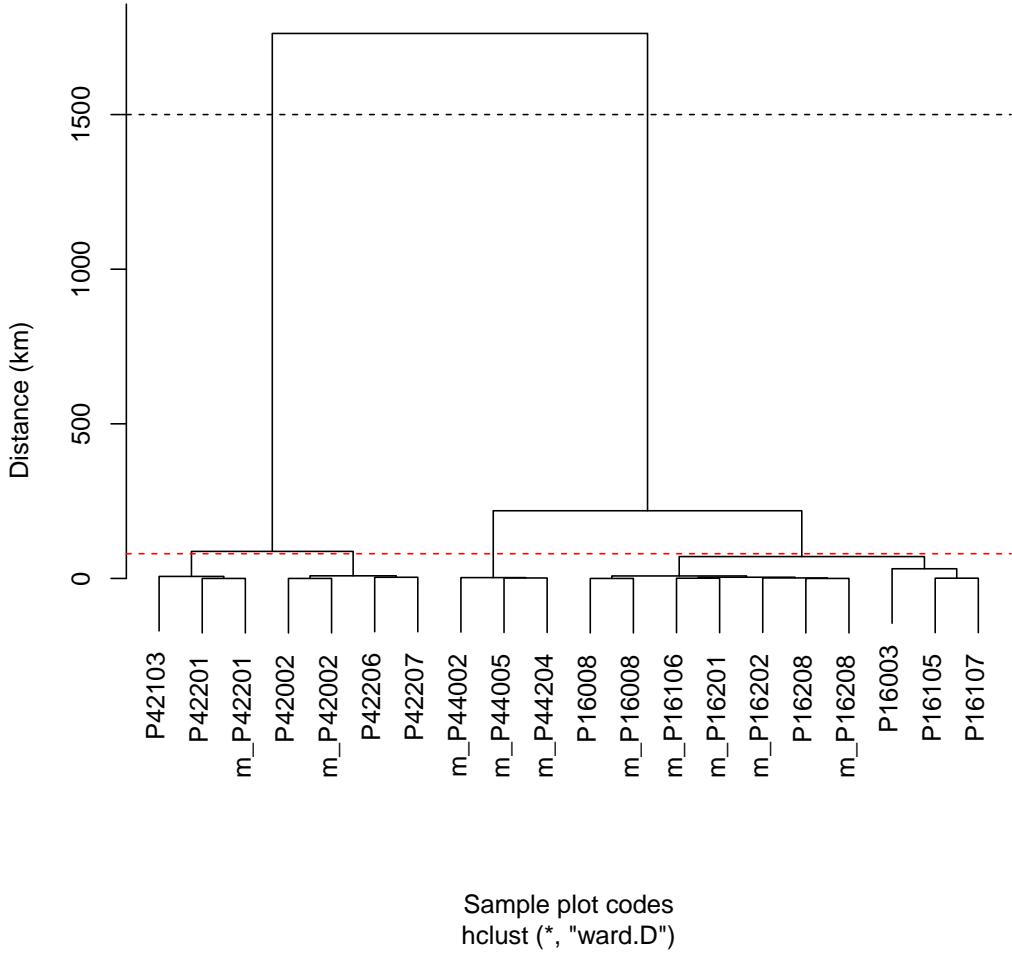


Figure 2: Relative geographic closeness between sample plots. Plots with master series are indicated with m\_. Codes of sample plots begin with initial letter of the species (*P. pinaster*); following with two digits in code indicating the province code: 42 corresponding to *Soria* on Northern portion of *Ebro* river basin, and 16 being *Cuenca* on Southern region of such a basin; last three digits in codes indicate individual number of the sample plot. Sample plots of master series have been indicated with the letter m\_. Black dashed line splits distance dendrogram in the two geographical portions of the river basin: North and South (distances > 1500 km); and the red dashed line defines four groups used to statistically control (cross-dating) dendrochronological series (distances < 80 km).

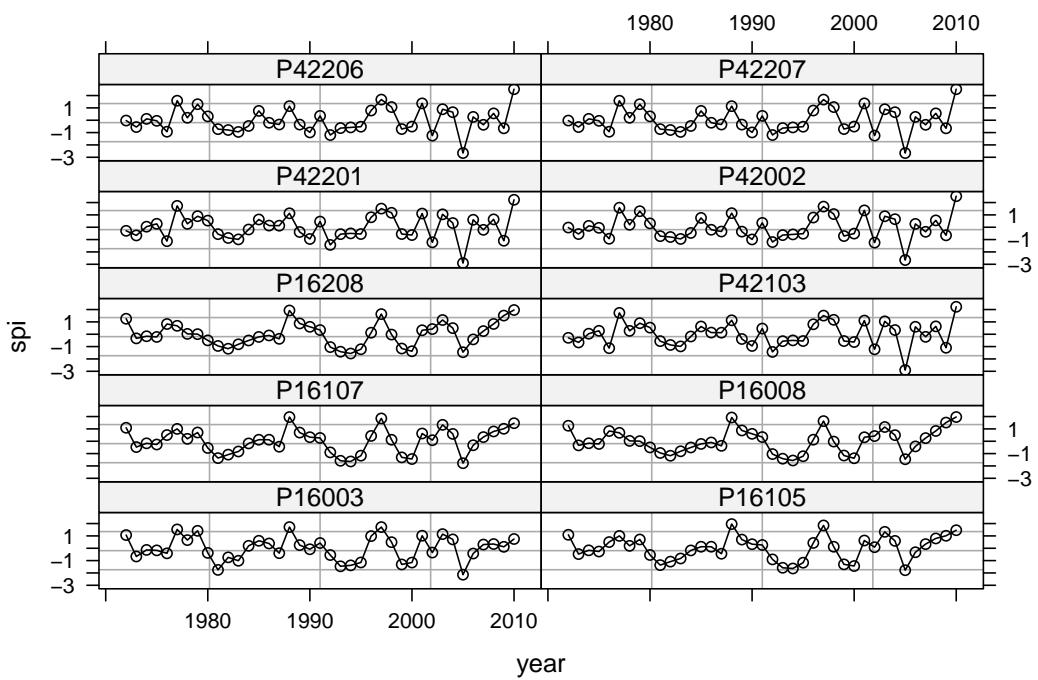


Figure 3: Series of standardized precipitation indexes (spi) on ten sample plots of *P. pinaster* located on Northern portion of *Ebro* river basin (42: *Soria*) and on Southern region of such a basin (16: *Cuenca*). Panels are ordered from plots with lower spi values (lower-left panel) to plots with higher spi extremes (higher-right panel). See legend in Figure 2 for further explanation of both: codes and plot locations.

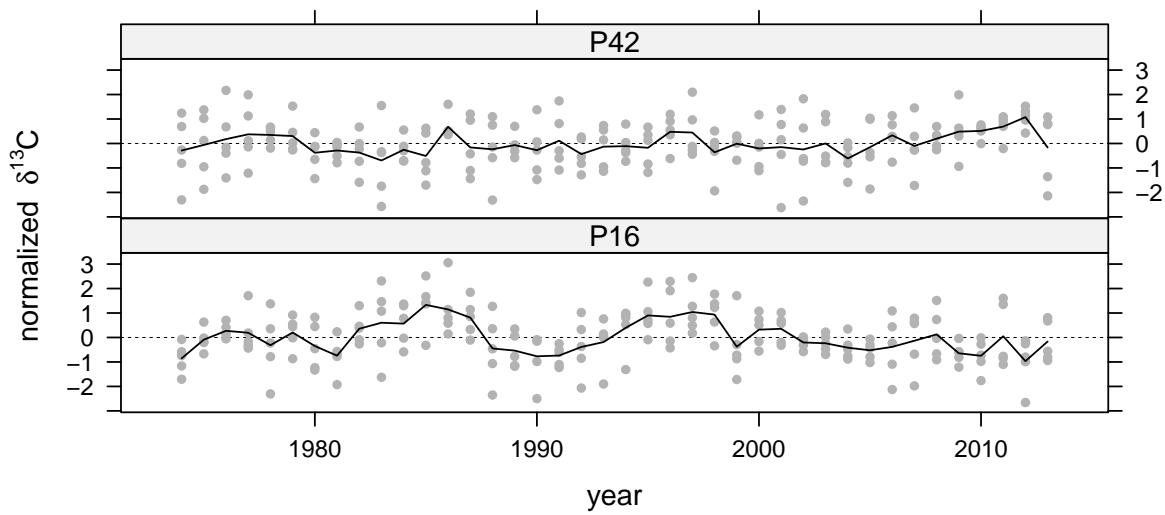


Figure 4: Normalized  $\delta^{13}\text{C}$  of *P. pinaster* late-wood in trees growing on Northern Ebro basin (P42) and Southern portion of the basin (P16), Spain

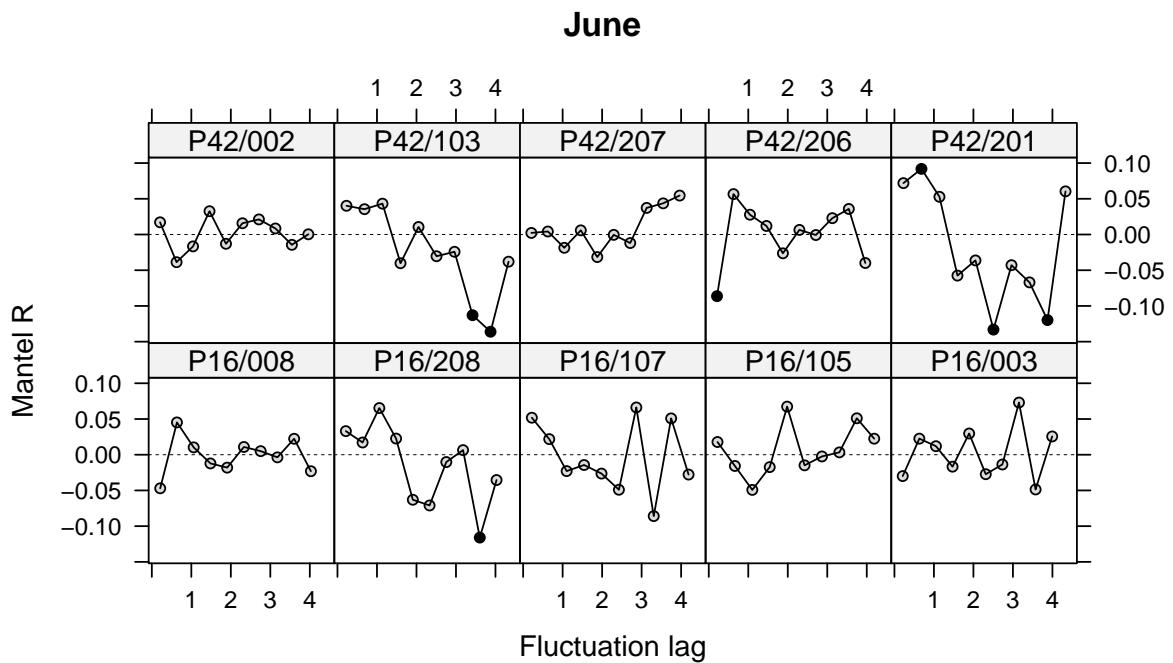


Figure 5: Signatures between normalized  $\delta^{13}\text{C}$  of *P. pinaster* late-wood and precipitation indexes (June) from 1974 to 2010. Signatures were computed with Multilevel correlograms. The Fluctuation lags were computed with Sturdges' rule.  $10^4$  permutation tests were developed on compared fluctuation-distance matrices.

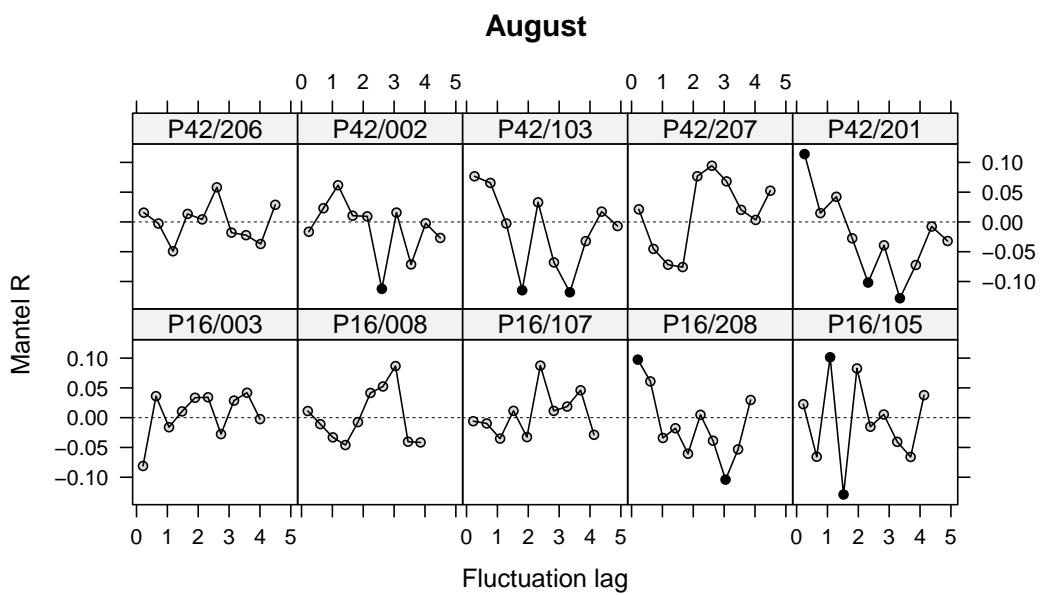


Figure 6: Signatures between normalized  $\delta^{13}\text{C}$  of *P. pinaster* late-wood and precipitation indexes (August) from 1974 to 2010. Signatures were computed with Multilevel correlograms. The Fluctuation lags were computed with Sturdges' rule.  $10^4$  permutation tests were developed on compared fluctuation-distance matrices.