

RESEARCH ARTICLE

Multilevel analysis of dendroclimatic series with the R-package `BIOdry`

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Abstract

The R-package `BIOdry` allows to model and compare fluctuations of Tree-ring Width (TRW) and climate, or dendroclimatic fluctuations, while accounting for source variability. The package eases multilevel modeling and multivariate comparison in dendroclimatic analysis using the `nlme` and `ecodist` packages, respectively. For implementing such libraries, the in-package algorithms transform the dendroclimatic fluctuations into Multilevel Dendroclimatic Data Series and maintain categorical variables and time units in the outputs. The dendroclimatic modeling is developed with two functions: `modelFrame` and `muleMan`. The first function binds core-level cumulative TRWs to the processed data sets and subtracts trends in TRWs by fitting multilevel log-linear growth formulas or multilevel linear formulas. `modelFrame` can also model within-group fluctuations in dendroclimatic variables other than tree-radial increments such as aridity indices or allometric components of tree growth: e.g. diameters at breast height over bark, tree basal areas, total tree biomass, among other. The second function compares fluctuations in `modelFrame` objects that share outermost categorical variable and annual records. Here, we use `BIOdry` to model dendroclimatic relationships in northern and east-central Spain to illustrate future users in the implementation of the package for modeling ecological relationships in space and time.

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1 Introduction

Dendrochronological studies date and analyze tree-rings from woody plants to understand past and current environmental processes. Dendroclimatology uses tree rings to study past climate conditions. It is one of the most prominent dendrochronological application to study global climate change [1]. Most standard approaches to model Tree-ring Width (TRW) and climate fluctuations implement time-series decomposition at specific levels of variance (single-level modeling) [2, 3]. The TRW data extraction usually requires sampling with specialized hardware [4] and/or specific statistical software [2, 3, 5, 6]. These procedures have been efficiently implemented not only for climate change studies [1] but also in paleoclimatic reconstructions [1, 7–9], the assessment of forest response to water availability [10, 11], among

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others [12–15]. Even though these methods are widespread applied, they have not always considered the sampling design and the different levels of variation in tree-ring series. Sampling dendroclimatic variables in forest ecosystems results in hierarchical sources of variability from ecological factors. For instance, variability in core replicates (number of samples per each analyzed tree) of TRW from forest communities would have at least three hierarchical levels: tree-radial morphology [16], individual-tree genetics or phenotype [17], and stand quality [15]. Additional ecological factors such as site elevation [18], fire intensity [14], tree decay [19], or water regimes [10, 11] can further complicate hierarchical variance in TRW. Meteorological records, which are used to model climatic variables in dendroclimatic analyses, also contain temporal and spatial variability [20, 21].

Developing software for dendroclimatic analysis in forest ecosystems can help to address these sources variability. This new software should account for the effects of both ecological-factors and sampling schemes, and should integrate tree-growth/yield modeling into dendroclimatic analysis [22–24] based on multilevel modeling and multivariate comparison.

One example of multilevel modeling methods is mixed-effects regression [25–28], which can be implemented to detrend dendroclimatic data by considering random effects from ecological factors. Another example is dissimilarity analysis [29–32], which can be used to compare and organize dendroclimatic fluctuations into common ecological-factor levels. However, the implementation of these methods using statistical environments requires dendroclimatic inputs stored in special formats [33] such as Multilevel Dendroclimatic Data Series (MDDS), or sequences of observations ordered according to spatial/temporal hierarchies which are the result of sampling schemes, with sample variability confined to ecological factors.

The dendroclimatic modeling in forest ecosystems should also integrate information about tree allometry, growth, and yield. For example, allometric scaling can help transform TRW data into other serial components of tree growth [34–38]. Similarly, organic growth theory has provided simplified equations [39–41] that can be used to subtract ontogenetic trends from TRW data. Such equations are log-linear expressions of tree growth that are easily fitted to the multilevel data applying mixed-effects-modeling procedures: e.g. `lme` methods in R [42].

In addition, dendroclimatic modeling can also involve time-series transformations at specific levels in the ecological factors. Such modeling is important for evaluating allometric parameters for TRW [36–38], computing water-availability indexes from climatic MDDS [20, 43–45], developing statistical process control for TRW series [2, 46–48], or for time-series smoothing and decomposition [49, 50]. Procedures to evaluate this kind of routines should be efficient and preserve MDDS structures and be adaptable to new methods in dendroclimatic modeling. Consequently, efficient implementation of all these procedures requires of programming higher-order functions which can recursively evaluate diverse routines, control multilevel detrending, and compare fluctuations in MDDS.

In this work, we present the R-package `BIOdry`, a statistical package that processes MDDS using higher-order functions to integrate processes for input derivation, multilevel analysis, and multivariate comparison of dendroclimatic fluctuations. Here, we explain the functionality of the package by modeling relationships between TRW fluctuations and drought using MDDS from pine forests in the Iberian Peninsula. We also test the hypothesis that accounting for ecological factors improves statistical analysis for dendroclimatic modeling compared to other conventional alternatives, such as linear regression. To do so, we compare parameters from implementing two procedures: conventional single-level modeling and the multilevel procedures in the package. The examples of dendroclimatic modeling developed here are also intended as a guideline for future `BIOdry` users interested in implementing the package for modeling other ecological relationships in time and space.

2 Package installation and requirements

The primary focus of this paper is on BIODry 0.5 (R versions $\geq 3.4.2$). This and further versions of the package can be easily installed in any R session using the `install.packages` command. Once installed, the package is loaded in the R environment with the `library` command. The in-package routines are programmed with generic functions in `base`, `stats` and `rutils` packages. Functions for developing the multilevel modeling and the multivariate comparison depend on two R packages in CRAN: `nlme` [42], and `ecodist` [30]. These are automatically loaded in R after the package has been requested. A further dependence, the `nlme` package, is used to extract fluctuations in MDDS, or Multilevel Dendroclimatic Data Series, with linear mixed effects models (detrending); while `ecodist` is used to compute multivariate correlations (Mantel correlograms) between two compared MDDS.

2.1 Data inputs

BIODry processes three kind of data sets: TRW (mm), monthly cumulative precipitations (mm), and monthly average temperatures ($^{\circ}\text{C}$). These can have wide or long formats. In the wide data format, the rows are ordered and labeled from earliest to latest year and the columns contain the dendroclimatic variables referred above. Names of the columns must be dot-separated codes representing the hierarchy of ecological factors, where higher ecological levels are defined first and lower levels after. For instance (truncations are indicated with ellipses):

```
> data(Pchron)
> tail(Pchron, 3)
      P16106.17.a      P16106.17.b... P44005.7.b
2003      0.926      0.882...      0.621
2004      1.205      0.638...      0.368
2005      0.964      0.295...      0.038
```

`Pchron` is a TRW (1861–2005) for *Pinus pinaster* Ait. forests (*P. pinaster*) in northern and east-central Spain [51]. These two regions have contrasting climate regimes with forests in central areas of the country being more affected by drought than forests in northern regions. To account for source variability, two trees were selected per site, and two cores were extracted from each tree. Consequently, the column names have within-plot levels representing variabilities in the plot qualities (alphanumerics), tree genetics/phenotypes (numbers), and tree-radial morphology (lowercase letters).

Meteorological records in wide formats have the rows labeled with years and the columns labeled with dot-separated codes of nearby plots and monthly abbreviations. For instance:

```
> data(Prec)
> data(Temp)
> names(Prec) # or names(Temp)
[1] "P16106.Apr" "P16106.Aug" "P16106.Dec" "P16106.Feb"...
```

The `Prec` sample data set, included in BIODry, consists of cumulative precipitations, while `Temp` contains corresponding monthly temperatures. These were provided by the Spanish Meteorological Agency (AEMET).

On the other hand, long data formats should have the chronologies and time units in the initial columns, followed by ecological factors. In the factor hierarchy, columns of lower time

units or classification factor levels are defined first, and columns of higher levels are specified later. For instance,

```
> data(Prings05)
> head(Prings05, 3)
      x year sample tree plot
1 0.698 1897      a   17 P16106
2 0.878 1898      a   17 P16106
3 0.842 1899      a   17 P16106
```

where `Prings05` is a long format of data stored in `Pcrhon`.

An alternative vector of tree radii can also be specified although this is not mandatory. In this case, the year of measurement of the radii should also be recorded. Until recently, core samples often lacked the initial rings of the pits producing truncated growths [22]; so these reference radii can be used to scale the cumulative growth. This vector should be named with dot separated codes and contain codes in the tree factor of the TRW.

```
> data(Pradii03)
> Pradii03
P16106.17 P16106.18 P44005.56 P44005.7
122.2343  139.7485  158.5184  114.9109
```

3 Package functionality

Dendroclimatic modeling has evolved from paleoclimatic sciences where the ecological variability is seen as noise [9]. Consequently, statistical procedures in dendroclimatic research traditionally consider ecological variability as irrelevant. These standard methods model signs by independently detrending TRW/climatic fluctuations (Fig 1, upper panels) and comparing the extracted signs with cross-correlation functions.

Procedures in the `BIOdry` package can complement these approaches by modeling and comparing dendroclimatic fluctuations while accounting for ecological variability (Fig 1, lower panes). To do so, the package implements methods to derive, detrend and correlate multilevel dendroclimatic fluctuations while accounting for factors defined in MDDS (Appendix A). These are programmed using a variety of in-package routines (Tables 1 and 2) which could be implemented independently if users are interested in running specific calculations or better understanding the sequence of steps for dendroclimatic modeling. However, implementation of these can be burdensome. Consequently, the routines can also be recursively evaluated on MDDS using two higher-order functions: `modelFrame` and `muleMan`. Former `modelFrame` can either derive allometric components of tree-growth fluctuation from TRWs data or compute aridity indexes from meteorologic records depending on whether defaults in the function are maintained or modified.

3.1 Modeling features

3.1.1 Function defaults. By default, the `modelFrame` function synchronizes, cumulates, and detrends TRW by evaluating three routines and a detrending formula (Table 3). The routines are defined in the `fn` argument and vectorized over factors in the `lv` argument. The synchronization circumvents biases causing artificial increases or decreases in the modeled tree growth: slow-grower survivorship, big-tree selection, among other [22] while enhancing convergence of parameters during the detrending procedure [42]. The cumulative TRW are used to model ontogenetic growth fitting detrending formulas [41]. These are specified with the

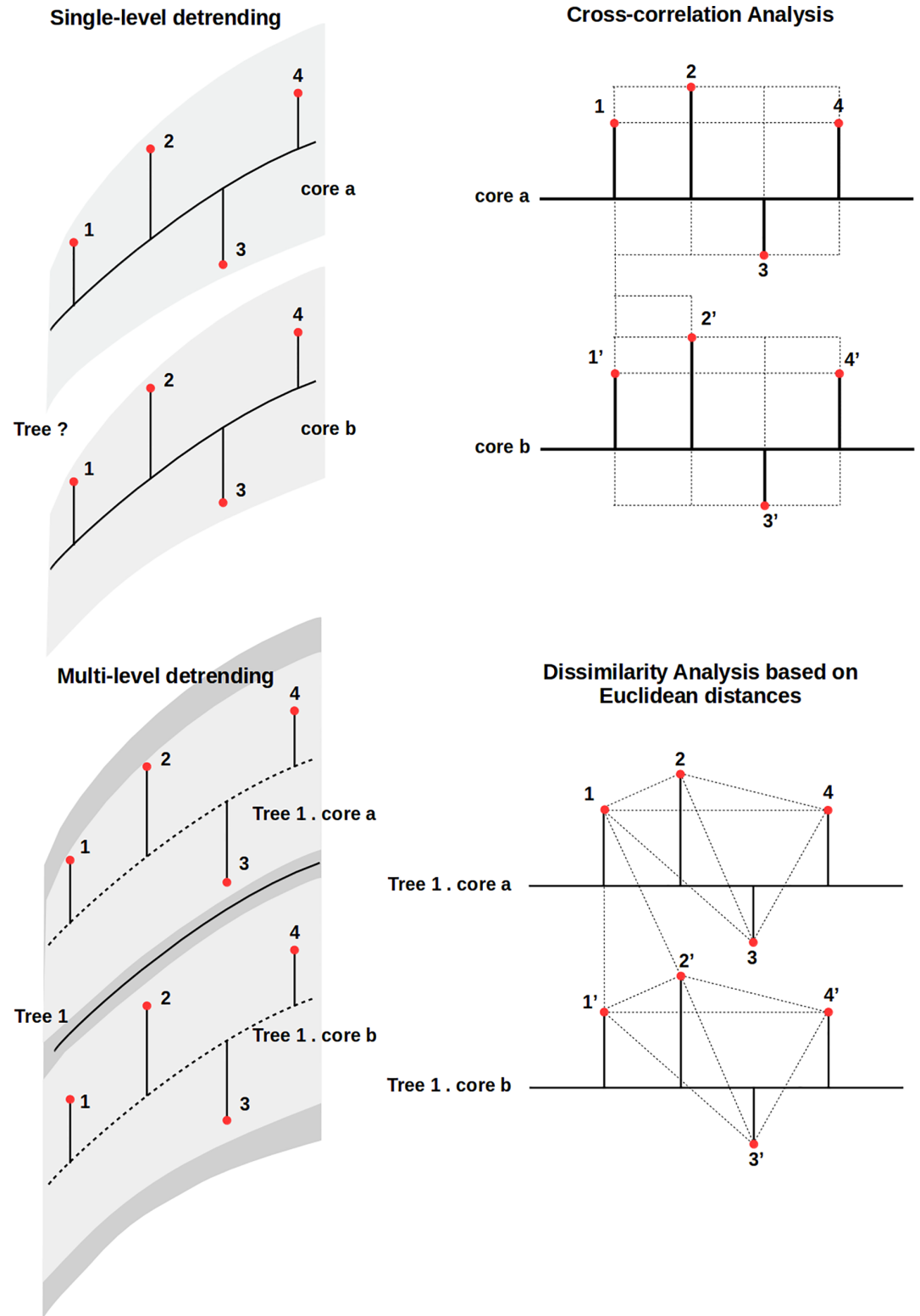


Fig 1. Comparison of procedures to model dendroclimatic fluctuations. Standard approaches (upper panels) usually account for sample variability (gray areas). These implement cross-correlation analyses to compare the detrended fluctuations. On the other hand, the BIODry package (lower panels) consider hierarchical structures from sample design (gray layers) to account for source variability: e.g. tree morphology. The package implements dissimilarity analysis to compare the detrended fluctuations.

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Table 1. Routines (rt) and detrending formula (df) used to model tree-growth fluctuations from TRW with modelFrame. These are specified in the form of lists with the `fn` and `lv` arguments (see second argument in Table 3). Arguments of these one-level functions are specified in `modelFrame` in either a `MoreArgs` list argument or directly, depending on whether they are vectors (e.g. `mp`) or constants, respectively.

Type	Name	Details	Arg.	Arg. definition
rt	<code>rtimes</code>	Unique observations in time-units data with replicates (time-series replicates) are excluded to avoid biases during subsequent multilevel detrending [22].	<code>only.dup = TRUE</code>	logical. Extract only relative times that are duplicated. If TRUE then unique observations are replaced with NA. If all computed times are unique then this argument is ignored. This function binds to the processed data set a new column <code>time</code> .
rt	<code>scacum</code>	Cumulative sums of time-series replicates (e.g. radial increments) are scaled on reference constants (e.g. individual tree diameters).	<code>sc.c = NA</code>	numeric. Scaling constant, or vector if the processed time-series replicates have several levels, to scale the computed cumulative values. If NA then the computed cumulative sums are not scaled.
			<code>rf.t = NA</code>	numeric. Constant or vector of reference time(s) in range(s) of the vector names to scale the cumulative values. If NA then maximum value in the range is used.
rt	<code>amod</code>	Simple allometric model: $y = a \cdot x^b$ is recursively evaluated to derive allometric components of organisms from longitudinal variables (e.g. Cumulative radial increments).	<code>mp = c(1, 1)</code>	numeric vector with allometric parameters: <i>a</i> , <i>b</i> . Default maintains the original radii, <code>c(2, 1)</code> produces diameters, and <code>c(0.25 * pi, 2)</code> computes basal areas. Further parameters of tree volume or biomass equations can also be implemented. This argument can have more than two parameters: <code>c(a1, b1, a2, b2, ..., an, bn)</code> , with <i>n</i> being the number of times that the allometric model is recursively implemented.
			<code>fun = y~a*(x^b)</code>	formula. Allometric model.
df	<code>tdform</code>	log-linear time-decline formula with random effects structure: <code>'log(x) ~ log(csx) + f(time) group'</code> ; where <i>x</i> is the relative organic growth; <i>csx</i> is the cumulative organic growth; <i>f(time)</i> is a function of time.	<code>on.time = TRUE</code>	logical. If TRUE then <code>t = 'time'</code> (see <code>rtimes</code> function in this Table). If false then <code>t = 'year'</code>
			<code>log.t = FALSE</code>	logical. If TRUE then <code>f(time) = log(time)</code> or <code>f(time) = log(year)</code> depending on the <code>on.time</code> argument.
			<code>lev.rm = NULL</code>	NULL or character vector. name(s) of the factor(s) to be removed from the <code>group</code> term.

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Table 2. One-level functions (rt) and detrending formula (df) used to model AAI fluctuations from monthly average temperatures and monthly cumulative precipitations. Implementation of these is similar to what was explained for tree growth modeling in Table 1.

Type	Name	Details	Arg.	Arg. definition
rt	<code>moveYr</code>	Monthly records in time-series replicates (usually of climate) are labeled for the years can begin in a month other than January.	<code>ini.mnt = 'Oct'</code>	character, or numeric from 1 to 12. Initial month of the seasonal year. If character then the months are built-in constants in R-package base. Default 'Oct' makes the years begin in October, for example.
rt	<code>wlai</code>	Annual aridity indexes from Walter-Lieth diagrams are computed from monthly precipitation sums and monthly average temperatures.	<code>sqrt = TRUE</code>	logical. Print the square root of the aridity index. If TRUE then computed aridity index is normalized with a square root transformation.
df	<code>lmeform</code>	LME formula with random effects structure: <code>'resp ~ cov group'</code> ; where <i>resp</i> is the response; <i>cov</i> is the primary covariate; and <i>group</i> is the random-effects structure.	<code>resp = NULL</code>	NULL or character. Column name of the response. If NULL then the name of the first numeric column in the MDDS is used.
			<code>covar = NULL</code>	NULL or character. Column name(s) of the covariate(s). If NULL then the name of the first time-units column in the MDDS is used.
			<code>lev.rm = NULL</code>	NULL or character vector. Name(s) of the factor(s) in the MDDS to be removed from the <code>group</code> term.

<https://doi.org/10.1371/journal.pone.0196923.t002>

Table 3. Formulation order of parameters in `modelFrame`. This function is used to model fluctuations of Tree-ring Widths (TRWs) (cm) and Annual Aridity Indexes (AAIs) (dimensionless).

Order	Arguments and defaults	Description
1	<code>rd</code>	Individual <code>data.frame</code> of TRW or twofold <code>list</code> of data frames with monthly cumulative precipitations and monthly average temperatures.
2	<code>fn = list('rtimes', 'scacum', 'amod')</code>	<code>list</code> character names of functions for one-level modeling (one-level functions) that are recursively implemented. See arguments in Tables 1 and 2.
3	<code>lv = list('tree', 'sample', 'sample')</code>	<code>list</code> . Either column names or numeric positions in the column factors in the MDDS that are used to evaluate the one-level functions.
4	Arguments and defaults in one-level functions	See one-level functions (<code>ofn</code>) and respective arguments in Tables 1 and 2.
5	<code>form = 'tdForm'</code>	Detrending formulas (Tables 1 and 2)
6	Arguments in <code>lme</code> function	

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`form` argument in `modelFrame`. The defaults are maintained by only specifying the processed MDDS. For instance,

```
> trwf <- modelFrame(Pchron)
```

On the other hand, `muleMan` compares TRW and aridity-Index fluctuations while maintaining hierarchical structures in the MDDS, see Section 7.1.

3.1.2 Detrending formulas. Currently, the `'tdForm'` or `'lmeForm'` methods can be implemented automatically with the `form` argument to evaluate formulas with the same names. The `'tdForm'` method is a linear generalization from growth theory (Appendix A.1) used here to subtract ontogenetic growth from TRW. This method considers either time units or classification factors in MDDS as random effects, see details about formulation of `'tdForm'` method in Section A.1.2. The `'lmeForm'` method implements a more flexible linear formula, which is implemented here to detrend normalized aridity indexes. See also structure of `'lmeForm'` method in Section A.1.3.

3.1.3 Correlation functions and variance structures. The package can implement procedures for serial normalization in the `nlme` package. Heteroscedasticity and serial autocorrelation of the detrended MDDS can be modeled with arguments in `lme` methods that are specified in `modelFrame`: e.g. weights and correlation arguments in the `nlme` package (Appendices A.3 and A.4). Likewise, autocorrelation in detrended MDDS can be assessed with empirical autocorrelation functions.

3.1.4 Multivariate comparison. Mantel correlograms between two MDDS with a common classification factor are established by comparing distances in one of the MDDS with sets of binary model matrices that specify membership in the other MDDS classes (Appendix A.5). The multivariate comparison is implemented here to compare detrended aridity indexes with detrended TRW.

4 Tree-growth modeling and diagnostics

Parameters of tree growth can be enhanced by changing arguments of the routines in the `modelFrame` function or updating `modelFrame` objects with the new arguments. The new model updating can help for properly scaling truncated fluctuation [22] and correctly defining shape of tree ontogeny [41]. TRW lacking initial rings in the pits can be scaled around the vectors of reference radii specifying the tree radii and corresponding measurement year in `sc.c`

and `rf.t` arguments, respectively. Likewise, the detrending formula can be modified from Chapman-Richards to Levakovic forms, changing default `log.t = FALSE` to `log.t = TRUE` respectively. The forms are simplified expressions of most growth equations used in forest sciences to model tree growth [41]. Here, we update the `trwf` object modifying these parameters with the update call:

```
> trwf <- update(trwf,
                 sc.c = Pradii03,
                 rf.t = 2003,
                 log.t = TRUE)
```

The `trwf` object contains a threefold list of model parameters in `model`, TRW fluctuations in `fluc`, and the model call in `call`. Parameters in `trwf` can be inspected calling the `model` element with currency or bracket operators and using plenty diagnostic functions in the `nlme` package [42]. Alternatively, we have developed five in-package S3 methods to directly inspect parameters in `modelFrame` objects with `lme` functions: `summary`, `plot`, `getData`, `anova`, and Empiric Autocorrelation Function (ACF). These are implemented here to inspect significances of fixed-effects parameters, compare models with similar fixed-effects structures, and detect serial patterns in normalized fluctuations.

4.1 Model diagnostics

The `summary` function provides information about the estimation method (default `method = REML`), information-based criteria (Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Log-Likelihood (`logLik`)), fixed-effects formula (default form = `'tdForm'`), random-effects standard deviations, and conditional t-tests, as shown in following summary output:

```
> summary(trwf)
Linear mixed-effects model fit by REML
Data: fixed
      AIC      BIC    logLik
1425.193 1488.797 -699.5963
Random effects:
Formula: ~log(csx) + log(time) | plot
Structure: Diagonal
      (Intercept)    log(csx)    log(time)
StdDev:    0.9541355 0.00115044    0.226334
Formula: ~log(csx) + log(time) | tree %in% plot
Structure: Diagonal
      (Intercept)    log(csx)    log(time)
StdDev:    0.872989 2.42083e-07    0.2220615
Formula: ~log(csx) + log(time) | sample %in% tree %in% plot
Structure: Diagonal
      (Intercept)    ~log(csx)    log(time)    Residual
StdDev:    0.09011541 6.108796e-24 0.02288596 0.4784995
Fixed effects: log(x) log(csx) + log(time)
      Value Std.Error  DF  t-value p-value
(Intercept) -3.171448 1.4210373 978 -2.231784 0.0259
log(csx)    1.398107 0.3585760 978 3.899055 0.0001
log(time)  -0.953834 0.2296603 978 -4.153239 0.0000
```



```

Correlation:
  (Intr) lg(cs)
log(csx) -0.822
log(time) 0.423 -0.521
Standardized Within-Group Residuals:
      Min          Q1          Med          Q3          Max
-6.9065514 -0.5052164  0.1119620  0.6316315  3.5025194
Number of Observations: 988
Number of Groups:
              plot          tree %in% plot
              2              4
sample %in% tree %in% plot
              8

```

The random effects of the summary output define the limits of the hierarchical levels and are expressed in standard deviations. The fixed effects show conditional t-tests testing marginal significances of fixed effects. These suggest that all the fixed-effects terms of multilevel model in `trwf` are significant ($p \leq 0.026$). We can infer that tree-growth parameters derived from the TRW conforms to the theoretical growth patterns implied by the Levakovic expression of `tdForm`. Diagnostic plots for assessing the quality of the fit are obtained using the method for the `plot` function.

```
> plot(trwf) ## Fig (2)
```

Accounting for the within-plot variability affected patterns between the TRW fluctuations (Fig 2). Fluctuations from trees in similar plots were more comparable than these in different plots. This could be explained by the different biophysical conditions of the plots, for instance: genetic adaptations of the populations, local variations in nutrients and water availability, competition [52].

To analyze improvements in model fitting by accounting for within-core variability, we ignore the grouping structure of the `tdForm` formula and fit a single linear regression without random effects to the TRW data. The model is fitted with `lm` regression in default R-package `stats`. The cumulative TRW are recycled from the `trwf` object with `getData` function. Parameters fitted with the new `lm` model are compared with parameters of the multilevel model in `trwf` doing a log-Likelihood ratio test(`logLik`) with the `anova` function.

```

> trwf1 <- lm(log(x) ~log(csx) + log(time),
              data = getData(trwf))
> anova(trwf, trwf1)
      Model df      AIC      BIC    logLik  Test  L.Ratio p-value
trwf    1 13 1425.193 1488.797 -699.5963
trwf1   2  4 1525.847 1545.418 -758.9235 1 vs 2 118.6543 <.0001

```

This suggests that accounting for within-core variability significantly reduces AIC, BIC, and `logLik` ($p \leq 10^{-4}$). Consequently, we assert that multilevel model in `trwf` fits better than the `tdForm` model with only fixed effects.

4.2 Allometric components of tree growth

Allometric relationships in components of tree growth other than TRW: e.g. tree diameters, basal areas, or tree biomasses, have not been fully explored to understand non-linear relationships between tree growth and drought [53]. The `modelFrame` function can help to explore such relationships by recursively evaluating allometric parameters in `mp` and formatting metric

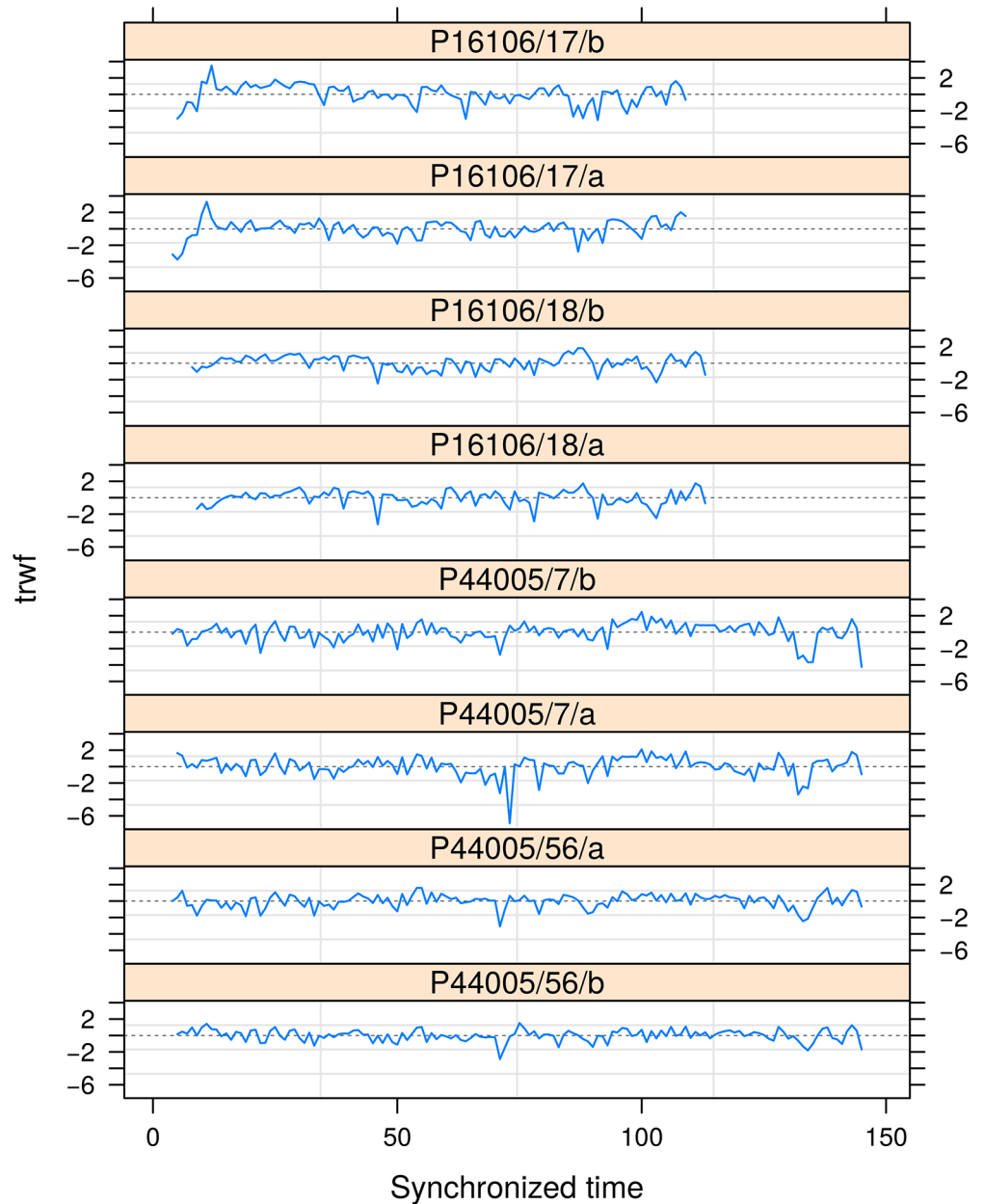


Fig 2. Fluctuations in the chronology of *P. pinaster*. Residual autocorrelations in the chronology has been accounted for with an auto-regressive structure for lags ≥ 2 (ARMA ($p = 1, q = 1$)) after normalizing the fitted residuals via Choleski factorization.

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units of the TRW in to argument. However, this recursive evaluation of allometric parameters must be done with care because excessive iteration of parameters could exponentially increase errors of the allometric models. Here, we specify two pairs of parameters in `mp` to compute fluctuations in Diameters at Breast Height over bark (DBH). The first pair of parameters `c(2, 1)` is used to compute diameters at breast height inside bark (`dib`) from the TRW. Metric units of the TRW are transformed from mm to cm before the `dib` are computed. This is done by writing `to = 'cm'`. The other two parameters `c(2.87, 0.85)` are used to

predict the DBH (cm) from the dib. The four allometric parameters are formulated in a common vector inside a `MoreArgs` list. This avoids the `modelFrame` function to vectorize over the allometric constants along the within-core levels.

```
> tdf <- update(trwf,
               to = 'cm',
               MoreArgs = list(mp = c(2, 1, 2.87, 0.85)))
> summary(tdf)
Linear mixed-effects model fit by REML
Data: fixed
      AIC      BIC    logLik
1425.903 1489.507 -699.9515
#...
Fixed effects: log(x) ~log(csx) + log(time)
              Value Std.Error  DF   t-value  p-value
(Intercept) -3.185970  1.3154323  978  -2.421995  0.0156
log(csx)      1.469242  0.4220967  978   3.480818  0.0005
log(time)    -0.954741  0.2298998  978  -4.152858  0.0000
#...
```

5 Random-effects structures

Hierarchical sources of variability from endogenous and exogenous disturbances in the stands are usually masked in the error terms of the TRW chronologies or the climatic fluctuations [54]. BIODry implements methods to account for this variability using the `modelFrame` function. By default, this function constructs random effects with `pdDiag` constructor class in `nlme` [42], a primary covariate from the formula in form, and the factors in the MDDS. However, users of the package can specify other constructor classes, see p. 157 in [42], and different random-effects covariates while removing factors from the processed MDDS. These are specified in either the `modelFrame` or `update` functions with the `random` and `lev.rm` arguments. We update here the `trwf` object with new parameters to modify original random-effects structure in `trwf` by specifying the `pdIdent` constructor class and the `~time` covariate while removing the sample random effect.

```
> trwfr <- update(trwf,
                 random = pdIdent(~time),
                 lev.rm = 'sample')
> summary(trwfr)
Linear mixed-effects model fit by REML
Data: fixed
      AIC      BIC    logLik
1510.969 1540.325 -749.4844
Random effects:
Formula: ~time | plot
Structure: Multiple of an Identity
              (Intercept)          time
StdDev: 8.272046e-07  8.272046e-07
```

```
Formula: ~time | tree %in% plot
Structure: Multiple of an Identity
          (Intercept)          time Residual
StdDev:  0.001311275    0.001311275 0.512138
#...
```

6 Serial modeling

Multilevel analysis of dendroclimatic data involves spatial and temporal pseudoreplication: i.e., series in the hierarchical levels are correlated introducing dependencies among the modeled fluctuations [55]. BIODry can print ACF plots to inspect such dependencies. The inspection process is controlled with two arguments: `maxLag` and `alpha`. The first argument controls the number of lags for which the autocorrelations are computed. The second argument specifies the significance level for two side critical bounds.

```
> plot(ACF(trwf, maxlag = 10),
       alpha = 0.01) ## Fig (3), upper panel
```

The plot shows that fluctuations in `trwf` are autocorrelated at three annual lags (Fig 3, upper panel). Part of this occurred because the core samples are replicated across the within-plot levels introducing dependencies among the residuals [55]. Another part of the autocorrelation could be explained by common climatic signals of the series [56].

The residual autocorrelation in multilevel models can be accounted for with standard `corStruct` classes in `nlme`, see p. 234 in [42]. Here, we account for the three annual-lag autocorrelation updating multilevel model in `trwf` with an auto-regressive structure for time lags ≥ 2 , see Appendix A.3. This is developed with `correlation` argument in the `update` function. Adequacy of the fitted correlation model is tested with diagnostic plots of ACFs applied to normalized fluctuations. Choleski decomposition of `lme` is implemented to normalize the fluctuations, see Appendix A.2. This is controlled with `resType='n'` argument in ACF. We also test improvements in parameters of the correlation model using the in-package `anova` method.

```
> trwfAC <- update(trwf,
                  correlation = corARMA(p = 1, q = 1))
> plot(ACF(trwfAC, maxlag = 10,
          resType = 'n'),
       alpha = 0.01) ## Fig (3), middle panel
> anova(trwf, trwfAC)
  Model df      AIC      BIC    logLik Test  L.Ratio p-value
trwf    1  13 1425.193 1488.797 -699.5963
trwfAC  2  14 1292.074 1360.571 -632.0371 1 vs 2 135.1184 <.0001
```

The ACF plot indicates fluctuations in `trwfAC` to be uncorrelated (Fig 3, middle panel), and the `anova` output evinces that accounting for the residual autocorrelation improves parameters of the multilevel model ($p \leq 10^{-4}$).

7 Aridity-index fluctuations

The Annual Aridity Index (AAI) is a good indicator of seasonal drought. This is constructed under the assumption that 1°C of monthly temperature amounts to 2mm month^{-1} of evaporation [57, 58] helping to identify periods of relative water surplus or deficit. `modelFrame` can model and detrend series of AAI processing the meteorological MDDS. Formulating

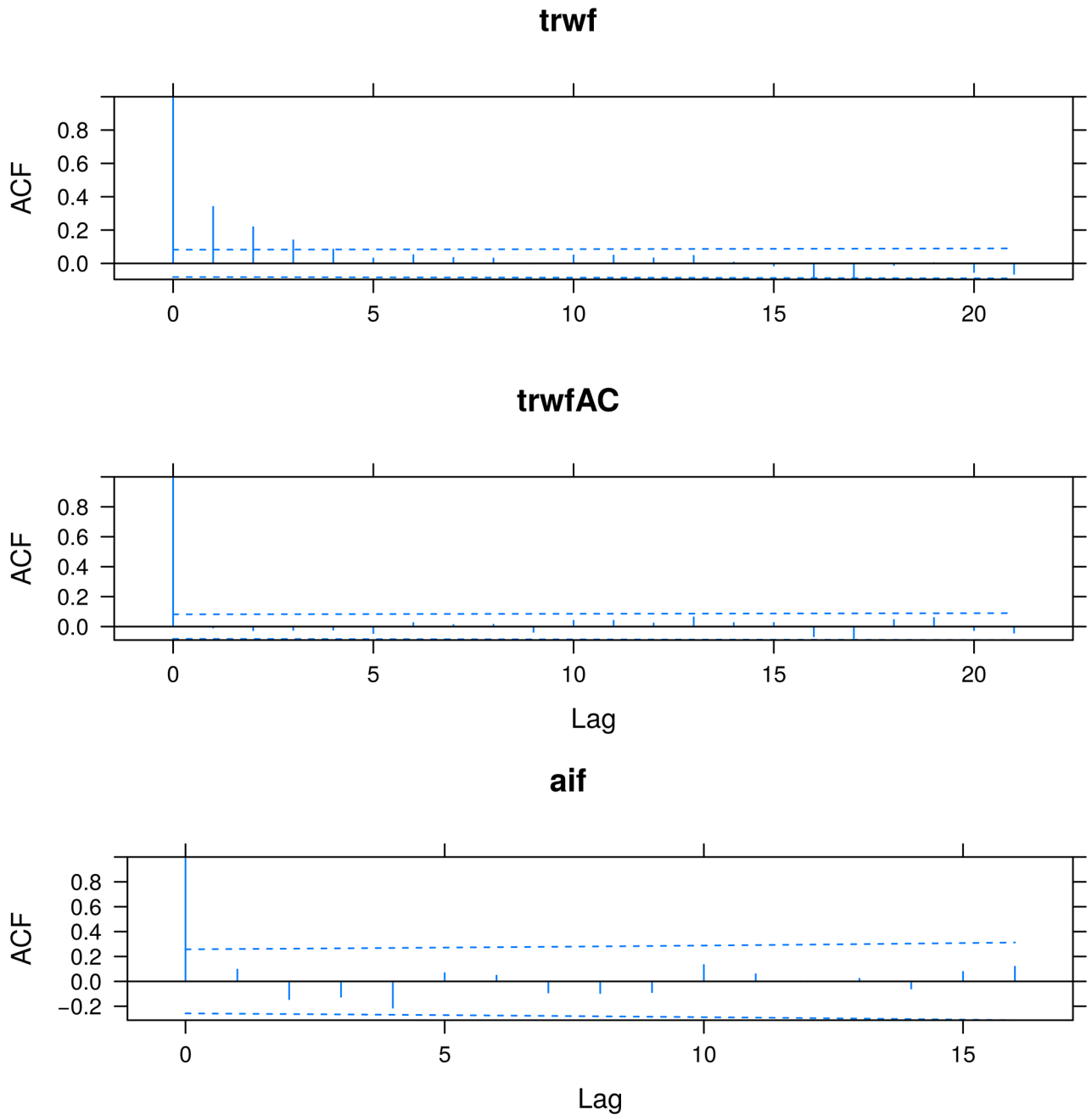


Fig 3. Empiric Autocorrelation Functions (ACFs) for the chronology of *P. pinaster* and the aridity-index fluctuations in three modelFrame objects. Labels indicate level-codes in the plot factor.

<https://doi.org/10.1371/journal.pone.0196923.g003>

parameters to model aridity indexes from Prec and Temp data sets using `modelFrame` is similar to what was explained for modeling tree-growth fluctuations. Here, new functions `moveYr` and `wlai` are specified in `fn` argument (Table 2). The `moveYr` function transforms time units into climatic variables for the years considered begin with October (default `ini.mnt = 'Oct'`, which is the beginning of meteorological year in Spain). The `wlai` function calculates square-root annual aridity indexes (default `sqrt = TRUE`). Both functions are evaluated at the year level with the `lv` argument. Fluctuations in the aridity-index are extracted using alternative `lmeForm` formula. This is a linear expression developed to detrend square-root aridity indexes, see Appendix A.1.3. Default Restricted Maximum Likelihood (REML) method is maintained.

```
> aif <- modelFrame(list(Prec, Temp),
  fn = list('moveYr', 'wlai'),
  lv = list('year', 'year'),
  form = 'lmeForm')
> summary(aif)
Linear mixed-effects model fit by REML
Data: fixed
      AIC      BIC    logLik
44.47932  57.40416 -17.23966
#...
Fixed effects: AI ~ year
              Value Std.Error DF  t-value p-value
(Intercept) -18.928112  3.648494  97 -5.187925      0
year          0.009985  0.001842  97  5.420766      0
#...
> plot(aif) ## Fig (4)
```

In this case, the fixed effects terms of the aridity-index model are significant ($p \approx 0$) suggesting that drought increased throughout the study area during the 50 years prior to sampling, even though the slope is very low (9.9×10^{-3}). Detrended plot-level aridity indexes fluctuate around zero over time and display constant variance (Fig 4).

Similarly to the TRW modeling, we compare parameters of the multilevel model in `aif` with parameters of an `lm` model without grouping structure. We also inspect presence of serial patterns in the aridity-index fluctuations.

```
> aifl <- lm(AI ~ year,
  data = getData(aif))
> anova(aif, aifl)
Model df      AIC      BIC    logLik  Test  L.Ratio p-value
aif    1  5 44.47932  57.40416 -17.23966
aifl   2  3 42.43269  50.18759 -18.21635 1 vs 2  1.953366  0.3766
> plot(ACF(aif, maxlag = 10, resType = 'n'),
  alpha = 0.01) ## Fig (3), lower panel
```

Parameters in the multilevel model in `aif` exhibit non-significant differences with regard to parameters in the `lm` model ($p \approx 0.38$) suggesting that more extensive meteorological records are needed to properly account for plot-level variability within the multilevel model. On the other hand, the ACF plot of normalized fluctuations in `aif` indicates no significant auto-correlations (Fig 3, lower panel). Considering that the fixed-effects parameters in `aif` are

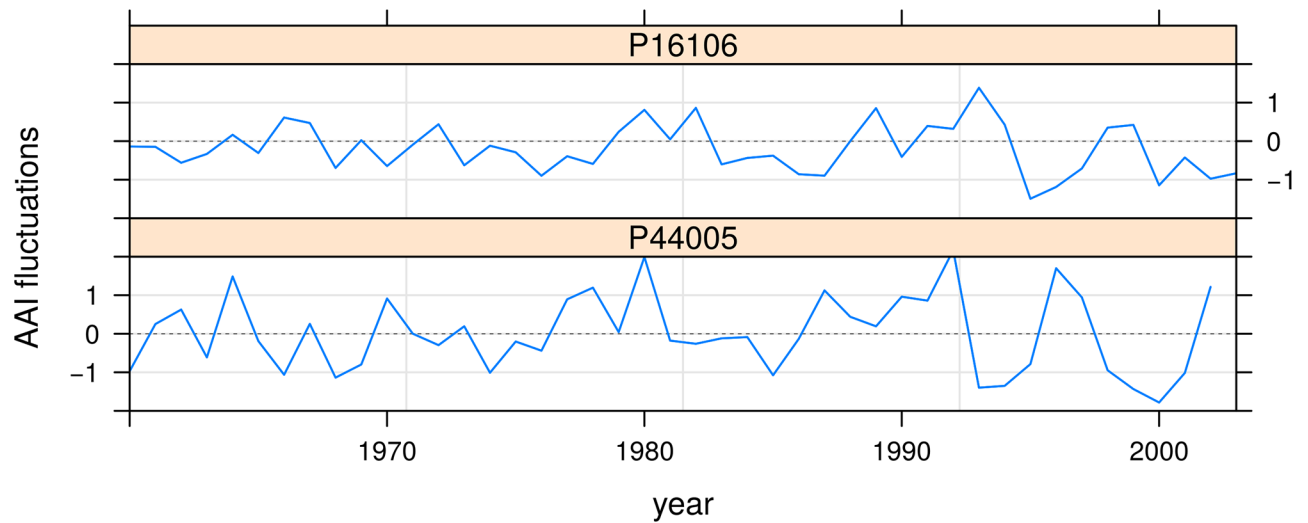


Fig 4. Normalized aridity-index fluctuations in aif object. Labels indicate level-codes in the plot factor.

<https://doi.org/10.1371/journal.pone.0196923.g004>

significant and that the corresponding fluctuations are not autocorrelated, we use the `aif` model during the final process of multivariate comparison.

7.1 Multivariate comparison

The BIODry package can measure relationships and patterns between classes of the dendroclimatic fluctuations by computing Mantel correlograms (Appendix A.5). This comparison is performed by evaluating the `muleMan` function. Here, we use this function to correlate dissimilarity matrices between fluctuations in the `trwf` and the `aif` objects which have common levels in the plot factor. We test significances of the multivariate correlations by repeatedly permuting the standardized dissimilarity matrices by rows and columns with the `nperm` argument.

```
> mcomp <- muleMan(trwf,
  cd = aif,
  nperm = 103)
> plot(mcomp) ## Fig (5)
```

The plot-level panels suggest that relationships between fluctuations in `trwf` and `aif` depend on the within-plot hierarchy, and plot location is the most important driver of dendroclimatic correlations (Fig 5). Within-plot variability affects the patterns of the computed correlations over time, while within-tree variability influences correlation trends and scales across the computed distance classes. Trends in the two sites exhibit oscillating pulses, and pulse frequency was significant for distance classes around 2 or 6. Although dendroclimatic interactions are stronger in East-Central Spain (P16106), they are more regular in Northern Spain (P44005) than in East-Central Spain (P16106). The Mantel correlogram also illustrates that dendroclimatic relationships are affected by within-core variability, such as radial-increment morphologies in this case. The core replicates of the same trees indicates both significant and no significant responses to drought (Fig 5, filled and empty circles, respectively), and discrepancies are more evident in P16106 than in P44005.

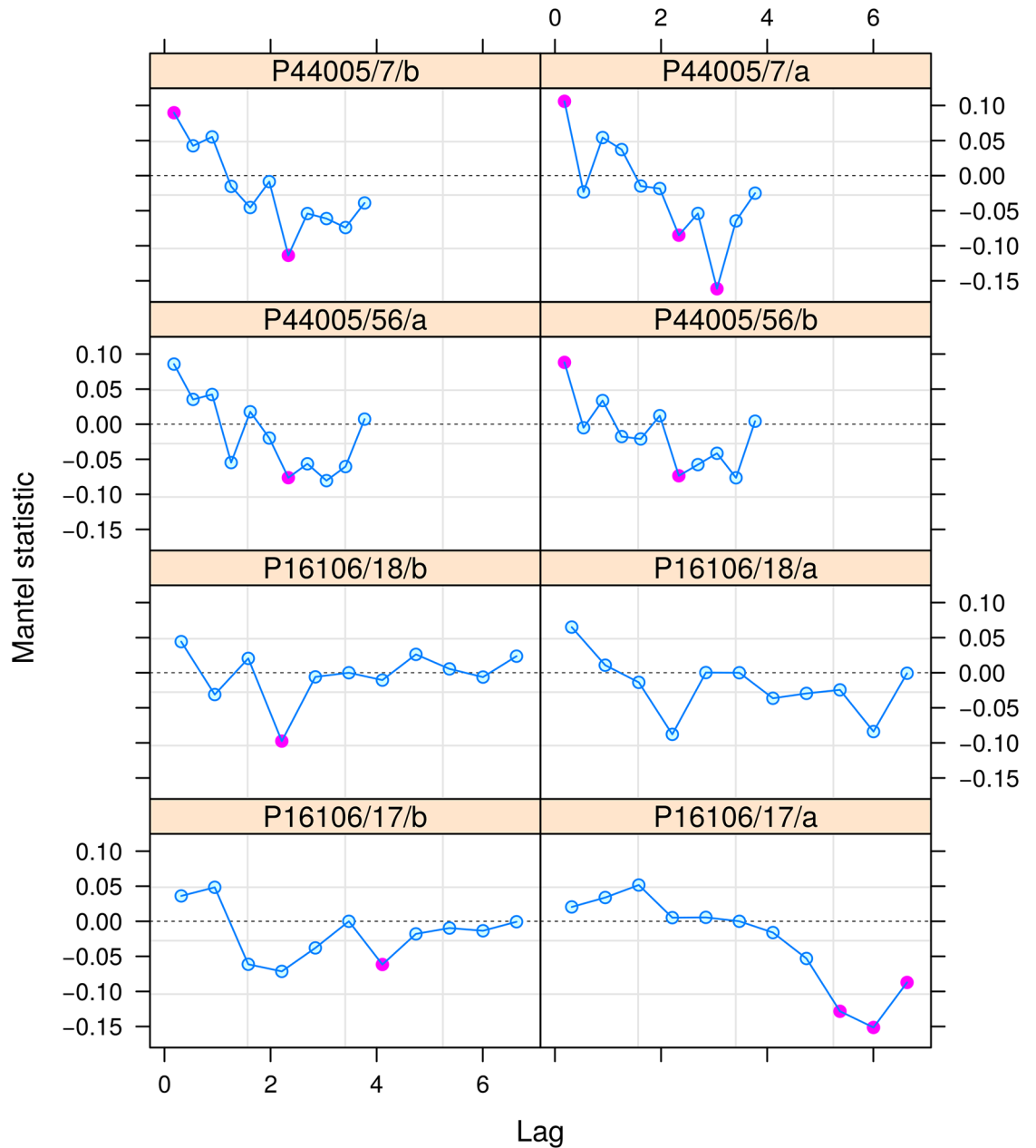


Fig 5. Multivariate correlations between the TRW chronology and the AAI fluctuations. Red circles indicate significant correlations ($p \geq 0.05$).

<https://doi.org/10.1371/journal.pone.0196923.g005>

8 Conclusion

The BIODry package is an useful tool for multilevel dendroclimatic modeling by integrating recursive evaluation of one-level functions, multilevel modeling, and multivariate comparison of dendroclimatic fluctuations. A larger community of researchers will find it relatively easy to use, as it only requires implementation of the `modelFrame` and `muleMan` functions. Most arguments in other functions of the same package, or in the `nlme` or the `ecodist` libraries, can be implemented with the same `modelFrame` and the `muleMan` functions. The

implementation of the functionalities within the `BIOdry` package to study the effects of dendroclimatic factors on forest-ecosystem dynamics could be greatly relevant for managing natural forests or monocultures, or for understanding the role of species mixtures in forestry. In the latter case, effects from dendroclimatic factors representing complementary resource uses (root stratification, nutrients, facilitative improvements, etc.) or even commercial productivity might be modeled with the package. We hope that other scientists can implement the `BIOdry` package to study the effect of such factors on many species and at different successional stages.

A Appendix: Multilevel and multivariate analyses with the `BIOdry` package

A.1 Trend subtraction

Currently, the R-package `BIOdry` has two detrending formulas: ‘`tdForm`’ and ‘`lmeForm`’. The first formula uses growth theory to subtract trends in organic variables. The second formula is a more flexible linear expression developed to detrend normalized aridity indexes and to formulate further linear relationships in MDDS: e.g. `gropedData` structures for panel plotting.

A.1.1 Growth theory. Ontogenetic component of organic growth (e.g. tree growth) behaves like a mechanistic open system with inputs and outputs of energy [40, 59, 60]. Diverse growth equations have been postulated on this basis to model, for instance, forest growth [39, 61–63], with most of the proposed equations being particular cases of following log-linear expression [41]:

$$\ln(Y') = \beta_0 + \beta_1 \ln(Y_t) - \beta_2 f(t) \tag{1}$$

where \ln is logarithm natural; Y' is the relative growth of the organism; β are parameters to be fitted; Y is cumulative organic growth; and t is the time. $f(t)$ is a function of time which can be $\ln(t)$ or t . When $f(t) = t$, Eq (1) is called a Time-Divide Form (TD), and when $f(t) = \ln(t)$ then the equation is a Logarithmic Time-Divide Form (LTD). Even though growth theory has provided theoretical balance equations, such as the TD or the LTD forms, to detrend tree growth series, conventional dendroclimatic software implements particular cases of growth or arbitrary equations, such as polynomials or even straight lines [2, 64]. These procedure has been successful in subtracting fluctuations and signals from dendrochronologic analysis [54]. Growth equations can help to enhance detrending process in dendrochronological research.

A.1.2 Multilevel detrending of MDDS. The ‘`tdForm`’ method in `BIOdry` package is a linear generalization of Eq (1) which considers either time units or classification factors in MDDS as random effects. This method function has the following structure:

$$\ln(Y'_{\dots ic, t}) = A_{\dots ic} + B_{\dots ic} \ln(Y_{\dots ic, t}) - C_{\dots ic} f(t) + \epsilon_{\dots ic, t} \tag{2}$$

$$A_{\dots ic} = \beta_0 + v_{\dots, 0} + v_{\dots i, 0} + v_{\dots ic, 0} \tag{3}$$

$$B_{\dots ic} = \beta_1 + v_{\dots, 1} + v_{\dots i, 1} + v_{\dots ic, 1} \tag{4}$$

$$C_{\dots ic} = \beta_2 + v_{\dots, 2} + v_{\dots i, 2} + v_{\dots ic, 2} \tag{5}$$

$$\epsilon_{\dots ic} \sim N(\mathbf{0}, \mathbf{R}), \tag{6}$$

where the β are the model parameters, $\mathbf{v} \dots = (v_{\dots, 0}, v_{\dots, 1}, v_{\dots, 2})^T$ are vectors of outermost levels of grouping, $\mathbf{v} \dots_i$ is a vector of tree random effects nested in the outermost levels of grouping,

and $\mathbf{v}_{...ic}$ is a vector of core random effects nested in trees that are nested in the outermost levels. The vector $\boldsymbol{\epsilon}_{...ic,t}$ contains the within-group residual errors $\epsilon_{...ic,t}$ and follows a multivariate normal distribution $\boldsymbol{\epsilon}_{...ic,t} \sim N(\mathbf{0}, \mathbf{R})$.

A.1.3 Trends in aridity indexes. Algorithms in BIODry can construct Walter-Lieth diagrams and compute annual aridity indexes as the ratios between dry and wet areas in the diagrams. The inputs are MDDS of monthly average temperatures ($^{\circ}\text{C}$) and monthly precipitation sums (mm). This integrates seasonal precipitations and temperatures to identify periods of relative water surplus and deficit [65]. Square root transformation of the aridity indexes are usually stationary [20]. Transformed aridity indexes also exhibit weak linear trends [52] that can be subtracted with simple linear equations, without further filtering the low and high frequencies observed in the raw precipitation and temperature records [43]. Autocorrelation in climatic proxies has been a frequent problem in dendroclimatology [47]. The square root transformed indexes use are weakly autocorrelated [52].

The 'lmeForm' method implements a flexible linear formula, which was developed to enable users formulate their own multilevel linear expressions. For instance, `form = 'lmeForm'` can be used to detrend the aridity indexes. In such a case, the 'lmeForm' method formula would have the following structure:

$$\sqrt{\mathbf{AAI}}_{k,t} = D_k + E_k \mathbf{t} + \boldsymbol{\omega}_{k,t} \tag{7}$$

$$D_k = \gamma_0 + \boldsymbol{\tau}_{k,0} \tag{8}$$

$$E_k = \gamma_1 + \boldsymbol{\tau}_{k,1} \tag{9}$$

$$\boldsymbol{\omega}_{k,t} \sim N(\mathbf{0}, \mathbf{R}), \tag{10}$$

where the γ are the model parameters, $\boldsymbol{\tau}_k = (\tau_{k,0}, \tau_{k,1})^T$ is the vector of location random effect. The vector $\boldsymbol{\omega}_{k,t}$ contains the location-level residual errors $\omega_{k,t}$ and follows a multivariate normal distribution $\boldsymbol{\omega}_{k,t} \sim N(\mathbf{0}, \mathbf{R})$. These are ordered on time and depict climatic fluctuations.

A.1.4 Back-transformation of model parameters. In some applications involving ecological analysis of dendroclimatic variables, it might be necessary to back-transform tree-growth and aridity-index predictions from Eqs (2) and (7) to the original scales. It is well known that exponential and power transformations could introduce biases and correction factors must be applied [66, 67]. However, it is not an objective of the BIODry package to find how correction factors could best be incorporated in mixed effects models. Analyses with the package focus on comparing dendroclimatic fluctuations without the need of back-transforming variables and the mixed-effects models include no correction factors. Therefore, inferences with the package apply only to the untransformed values of the parameters.

A.2 Residual normalization

Multilevel fluctuations in `modelFrame` objects are normalized via Choleski decomposition of the residual variance-covariance matrix, see p. 239 in [42]. For the innermost level of Residuals of Annual Biomass Increment (RABI) the normalized residuals are defined as follows:

$$\boldsymbol{\epsilon}_{\text{norm}, c, t} = (\mathbf{C}_{c, t}^T)^{-1} (\mathbf{y}_{c, t} - \mathbf{X}_{c, t} \hat{\boldsymbol{\beta}}), \tag{11}$$

where \mathbf{C}_i^T is the lower triangle of the Cholesky decomposition of the variance-covariance matrix \mathbf{V}_i , \mathbf{X}_i is the design matrix, $\hat{\boldsymbol{\beta}}$ contains the parameter estimates and \mathbf{y}_i is the vector of observed responses.

A.3 Autocorrelation function

Autocorrelation of normalized fluctuations in multilevel models is assessed with ACF, see p. 183 in [28]. As an example, the autocorrelation model for the lowest level of $\epsilon_{\dots ic,t}$ normalized residuals is

$$\hat{\rho}(l) = \frac{\sum_{c=1}^{n_i} \sum_{t=1}^{n_c-l} \epsilon_{c,t} \epsilon_{c,(t-l)} / N(l)}{\sum_{c=1}^{n_i} \sum_{t=1}^{n_c} \epsilon_{c,t}^2 / N(0)} \tag{12}$$

where l is the lag in years, and $N(l)$ is the number of residual pairs defining the numerator of $\hat{\rho}(l)$. Any correlation structure of nlme package can be implemented in modelFrame function to model residual autocorrelation. For the case of dendroclimatic data, autocorrelation in MDDS is modeled with an autoregressive-moving average models ARMA (1,1), which have exponentially decaying auto-correlation functions for lags ≥ 2 . For the lowest level of the $\epsilon_{\dots ic,t}$ normalized residuals the autocorrelation model is

$$\epsilon_{c,t} = \phi \epsilon_{c,(t-1)} + \theta a_{c,(t-1)} + a_{c,t}, \tag{13}$$

where ϕ was the autoregressive parameter; θ was the moving average parameter; and $a_{c,t}$ was the noise term, see p. 128 in [50].

A.4 Variance function

Heteroscedasticity and serial autocorrelation of $\hat{\epsilon}_{\dots ic,t}$ and Residuals of Annual Aridity Index (RAAI) can be modeled with correlation and weights arguments [42] in modelFrame function. However, such modeling would take a long time depending on the complexity of the classification factors in the MDDS. For example, the variance model for the innermost level of $\epsilon_{\dots ic,t}$ normalized residuals is:

$$\text{Var}(\epsilon_{c,t}) = \sigma^2(\varrho_1 + |v_{c,t}|^{\varrho_2})^2, \tag{14}$$

where σ^2 was the variance; ϱ_1 and ϱ_2 were constants; $v_{c,t}$ had the same structure of the fixed effects from Eqs (2) and (7).

A.5 Multivariate comparison

Mantel correlograms between two MDDS with a common classification factor are established by comparing distances in one of the MDDS with sets of binary model matrices specifying membership in particular classes of the other MDDS. Euclidean distances from both MDDS are standardized to z-scores by subtracting the means from individual distances and then dividing the differences by the corresponding standard deviations. The Mantel statistic is

$$r(d) = \frac{\sum_i^n \sum_j^n w_{ij} z_{ij}}{\sum_i^n \sum_j^n w_{ij}} \tag{15}$$

where d is the distance class from one of the MDDS; z_{ij} is the distance between each pair i and j from the other MDDS; w_{ij} is a weight for the pair: typically 1 if z_{ij} is in d and 0 if it is not. The number of classes d is calculated with the Sturges rule [29]:

$$d = 1 + 3.3 \cdot \log_{10}(m) \tag{16}$$

where m is the number of distances in the upper-triangular binary model matrix.

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