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The use of STATICO and COSTATIS, two exploratory three-ways analysis methods: an application to the ecology of aquatic heteroptera in the Medjerda watershed (Tunisia)

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Abstract This article is intended as a guideline to the use of two exploratory data analysis methods, namely STATICO and COSTATIS. Both techniques have already been used in the field of ecological data analysis, and we present a rapid survey of the ecological literature on three-ways analysis methods. Here, we wish to share some advanced computation and graphical display scripts to help ecologists use these methods. We first recall the main principles of these two methods for the analysis of the relationships between the structures of two series of data tables. In the context of ecology, these two series can be for example (1) a series of species data tables and (2) a series of environmental parameters tables. A detailed, real-size example is presented to show how this strategy can be put in place using the **ade4** and **adegraphics** packages for **R**. This example relates to the ecology of aquatic Heteroptera in the Medjerda watershed (Tunisia). We show how the outputs of the two methods can be used to

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interpret the relationships between aquatic Heteroptera species distribution and environmental parameters. Several **R** scripts to conduct the computations and draw suitable graphical displays are reproduced and explained in the text and in five appendices.

Keywords Ade4 · Aquatic Heteroptera · Costatis · Medjerda · Statico · Three-ways data analysis

1 Introduction

Classical multivariate data analysis methods are used to analyse one single data table. In ecology, this can be a species table (species in columns, sites in rows) or a table of environmental parameters (physico-chemical variables). The analysis of the relationships between these two kinds of tables (species tables and environmental parameters tables) is a key area of ecological data analysis. It calls upon another class of methods, called "coupling methods", like Canonical Correspondence Analysis, Redundancy Analysis, or Co-inertia analysis.

Three-ways multivariate data analysis methods provide a way to analyse a series of tables as a whole. The repetitions in a series can correspond for example to space, or time. These methods are used in ecology to analyse series of species data tables, or series of tables of environmental parameters. They provide information about the stability or the diversity of the structures common to all the tables of the series.

A further step consists in analysing the relationships between the structures of two series of data tables: one series of species data tables and one series of environmental parameters tables. This can be very useful to assess the stability of species-environment relationships. It can be used for example in conservation ecology or global change studies.

STATICO (Simier et al. 1999; Thioulouse et al. 2004) and COSTATIS (Thioulouse 2011) are two examples of three-ways data analysis coupling methods, but many other methods have been developped in the 2000s, particularly in the area of chemometrics. These methods are often called "multi-block" methods, and they are generalizations of three-ways methods. A review of all these methods have recently been presented by Abdi et al. (2012).

For example, the DO-ACT (Vivien and Sabatier 2004) is a generalization of the STATIS strategy (Lavit et al. 1994) to the case of two series of tables, and the GOMCIA (Vivien and Sabatier 2003; Vivien and Sune 2009) is a generalization of Multiple Co-Inertia Analysis (Chessel and Hanafi 1996). STATIS-4 (Sabatier and Vivien 2008) is a generalization of the STATIS strategy to the case of several series of tables. (Smilde et al. 2000) present a general theory of multiway multiblock component methods.

STATICO and COSTATIS are designed to analyse the relationships between two series of tables: one series of species data tables, and one series of environmental parameters tables. A species data table is a table with p species in columns and n sites in rows, containing the number of individuals (or an abundance index) of species j found at site i. An environmental parameters table is a table containing q environmental characteristics like for example physico-chemical parameters (columns), measured in the same n sampling sites (rows). Series of such tables can come from the repetition of sampling campaigns (in time), or from sampling several regions (in space).

Analysing such datasets globally can be a complex task, and methods like STATICO and COSTATIS provide convenient ways to extract and sum up the main characterictics of their structures. In this paper, we show how to use the **ade4** (Dray and Dufour 2007; Thioulouse and Dray 2007) and **adegraphics** (Dray and Siberchicot 2015) packages to do this. We detail and explain the functions performing computations and graphical display.

We use these functions to analyse a real-size example related to the ecology of aquatic Heteroptera in the Medjerda watershed (Tunisia). These aquatic and semi-aquatic insects, often referred to as "water bugs", are composed of two monophyletic infraorders, Nepomorpha and Gerromorpha, with 50 genera and 334 species in Palearctic region (Aukema et al. 2013), among which 13 families, 19 genera and 56 species occur in Tunisia (Slimani et al. 2015, 2016).

Water bugs are an important group of insects in various aquatic ecosystems due to their high density and various ecological functions (Cummins and Merritt 1996). Distribution and abundance of water bugs is related to the physicochemical conditions of water (Savage 1982; Hufnagel et al. 1999; Karaouzas and Gritzalis 2006; Carbonell et al. 2011). Families of water bugs differ considerably in morphology and ecological preferences, and many species display specific habitat preferences (*i.e.*, Corixidae) (Macan 1938, 1954; Savage 1990, 1994; Tully et al. 1991). They are found along the margins of shallow water (Micronectidae, Corixidae), moist places at shorelines, and floating plants (Hebridae, Veliidae Mesovelia), on the water surface of lentic (pool) streams (Mesoveliidae, Hydrometridae, Gerridae and Veliidae) and lotic (riffle) streams (some Veliidae), in aquatic vegetation (Nepidae, Notonectidae, Naucoridae, and Pleidae). They may also be found under rocks in running waters (some Naucoridae) and moist edge of rivers and on sand (Ochteridae).

All the computations and figures of this paper can be reproduced using three supplementary files. Two data files are available, one for environmental parameters: file "`Env.txt`" with 144 rows and 10 columns, (named "MOESM1" in the supplementary material) and one for water bugs: file "`Het.txt`" with 144 rows and 18 columns (named "MOESM2" in the supplementary material).

The complete **R** code to reproduce computations and figures is available in file "`Scripts.R`" (named "MOESM3" in the supplementary material). This file can be executed directly with the "`source()`" function of **R**. There is a large number of output formats for graphic files in **R**. Vectorized formats (e.g., pdf) can be used when some modifications need to be made on final figures.

2 Methods and data

2.1 Statistical methods

STATICO and COSTATIS are based on two pre-existing data analysis methods: Co-inertia Analysis (COIA, Dolédec and Chessel 1994; Dray et al. 2003) and Partial Triadic Analysis (PTA, Thioulouse and Chessel 1987; Kroonenberg 1989; Thioulouse 2011).

Co-inertia Analysis is, like Canonical Correspondence Analysis (Braak 1986) and Redundancy Analysis (Van Den Wollenberg 1977), a two-tables coupling method.

This means that it aims at exploring the relationships between two data tables. More precisely, COIA finds two sets of axes of maximum covariance that are linear combinations of the variables of the two tables (Dolédec and Chessel 1994; Dray et al. 2003).

The first step of COIA consists in computing the cross-covariances table between the variables of the two data tables. This cross-covariances table is then analysed and gives the Co-inertia axes. In ecology, coupling methods are used to analyse the influence of environmental parameters on the distribution of species, or reciprocally, to check the ability of species to be used as indicators of particular environmental conditions.

Partial Triadic Analysis is a STATIS-like (Lavit et al. 1994) three-ways data analysis method. It can be used to analyse a three-ways table, *i.e.*, a series of k tables. PTA is based on the concepts of vector variance and vector covariance (Escoufier 1973). But unlike STATIS that computes RV coefficient between operators (variable or individuals), PTA computes RV coefficients between data tables. Like other STATIS-based three-ways tables methods, PTA consists of three steps, namely Interstructure, Compromise and Intrastructure.

The first step (Interstructure) computes weights that are used to build a linear combination of the series of tables, called the "Compromise". These weights are the components of the first eigenvector of the RV coefficients matrix (see Lavit et al. 1994). The Compromise has the same structure (rows, columns) as one table of the initial series. In the second step, this Compromise is analysed using a Principal Component Analysis (PCA). The third step (Intrastructure) is the projection of the rows and columns of each table of the series in the analysis of the Compromise (Thioulouse and Chessel 1987; Thioulouse 2011). These methods are used in ecology to analyse series of species data tables. They provide information about the stability or the diversity of the structures of all the tables of the series.

As explained in Fig. 1 (left part), STATICO is the PTA of the series of cross-covariance tables obtained by crossing the variables of each pair of data tables. COSTATIS (right part of Fig. 1) is the COIA of the two Compromises obtained by the PTA of each of the two series of tables separately.

A rapid survey of the ecological literature shows that these three-ways methods are gaining more and more interest: since 2001, 14 papers were found using PTA, in the areas of earthworms biology (Decaëns and Rossi 2001; Decaëns et al. 2009; Jiménez et al. 2006; Rossi 2003), Phyto- and zoo- plancton (Bertrand and Maumy-Bertrand 2010; David et al. 2012; Hernández-Fariñas et al. 2014; Napoléon et al. 2012; Rolland et al. 2009; Mendes et al. 2010), water pollution (Gourdol et al. 2013; Jiménez et al. 2015), fish communities (Erős et al. 2012), and landscape ecology (Ernoult et al. 2006).

Since 2006, six papers used the STATICO method (Gonçalves et al. 2012; Marques et al. 2011; Mendes et al. 2009; Kidé et al. 2015; Carassou and Ponton 2006; Certain et al. 2011) and one paper used the COSTATIS method (Ladhar et al. 2015).

2.2 Biological data

2.2.1 Study area

The Medjerda watershed study area (Fig. 2) is an important hydrographic basin of Tunisia and Algeria. It drains an area of 23,500 km^2, of which 15,900 km^2 are in

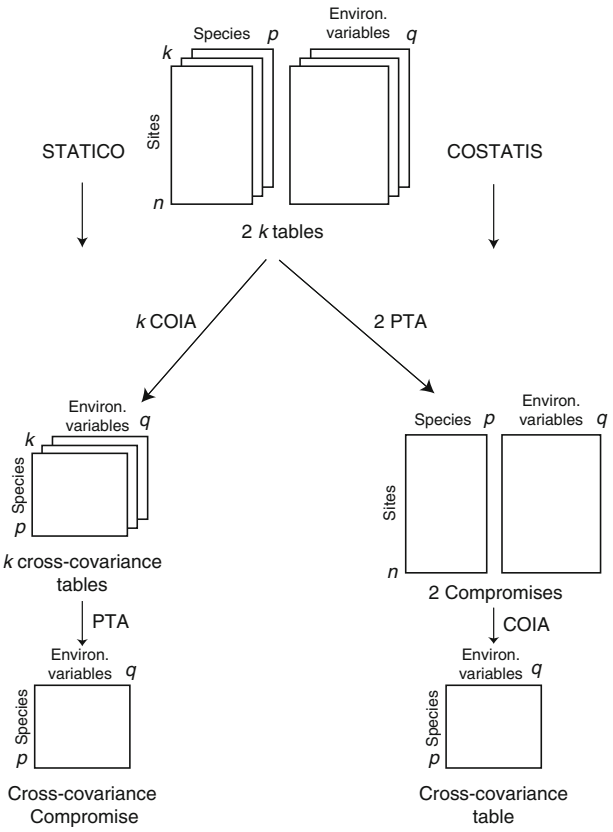

Fig. 1 Presentation of the STATICO (*left*) and COSTATIS (*right*) methods: STATICO is the PTA of the series of cross-covariance tables obtained by crossing the variables of each pair of data tables. COSTATIS is the COIA of the two Compromises obtained by the PTA of each of the two series of tables separately

Tunisia. The Medjerda and its tributaries collect most of the surface waters of northern Tunisia (Jaouadi et al. 2012). It crosses several urban areas in Algeria and Tunisia.

The Medjerda belongs to the Tellian domain and consists of a Quaternary depression limited by the nappe zone to the North (Ben Ayed 1986; Rouvier 1977) and the diapiric zone to the South (Perthuisot 1978; Ghanmi 1980). The central Medjerda basin in Northern Tunisia is a depression zone running in a west-east direction, and has its origin in the Atlas orogenesis (Faust et al. 2004). The middle Medjerda valley belongs to the Tellian zone of Tunisia. It is a post-orogenic basin where surface structural indicators are almost completely absent. Alpine and Atlasic tectonic prints are well expressed on both sides of the basin (Amiri et al. 2011).

The average annual temperature and mean annual precipitation in the basin is 17.8 °C and 462 mm, respectively (Faust et al. 2004). With hot and dry summer and rainy winter, the climate corresponds to the Mediterranean subtropics (Dungan et al. 2002). The region is characterized by the transition from Mediterranean semi-humid to semi-arid conditions (Faust et al. 2004). This transition is ecologically marked by the occurrence of the Mediterranean xerophytic forest with *Pinus halepensis* Miller and

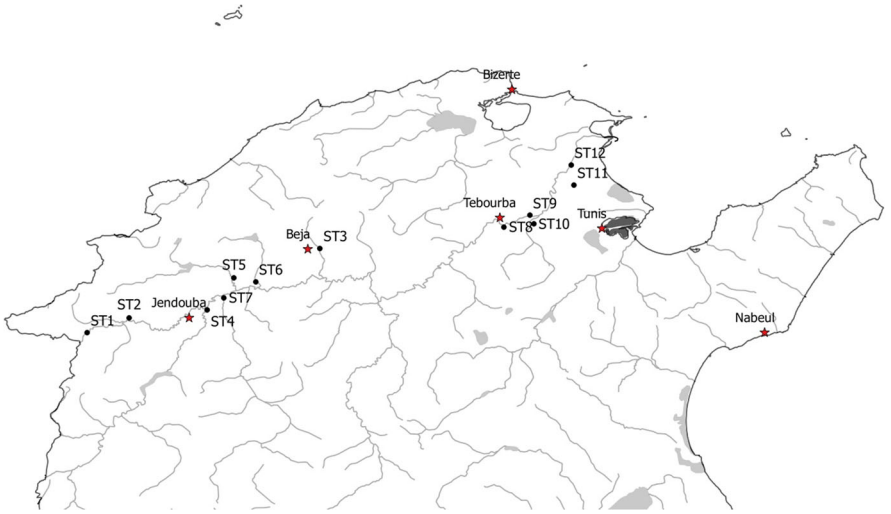

Fig. 2 Map of the study area showing the North of Tunisia, with the 12 sampling sites in the Medjerda watershed

Table 1 List of sampling sites

Code	Location	GPS	Altitude (m)
ST1	Chardimou	36°27′01.87″N−08°26′01.56″E	197
ST2	Chemtou	36°30′00.38″N−08°34′33.23″E	173
ST3	Beja	36°44′11.04″N−09°13′25.15″E	147
ST4	Mellegue	36°31′42.18″N−08°50′28.93″E	136
ST5	Kasseb	36°38′05.44″N−08°55′53.35″E	131
ST6	Bouhertma	36°37′22.90″N−09°00′17.52″E	130
ST7	Tessa	36°34′05.91″N−08°53′51.98″E	127
ST8	Battan	36°48′29.99″N−09°50′53.43″E	24
ST9	Jedeida	36°50′52.00″N−09°56′05.03″E	23
ST10	Chafrou	36°04′54.67″N−09°56′54.62″E	18
ST11	Khlaidia	36°57′02.71″N−10°05′06.72″E	5
ST12	Klaat Andalous	37°01′07.45″N−10°04′33.27″E	2

shrubs of *Quercus ilex* Linnaeus. Due to strong anthropogenic pressure, xerophytic forest is found only in recessional zones or protected areas.

2.2.2 Biological dataset

Aquatic bugs were sampled monthly from January to December 2013 in 12 locations of permanent streams in the Medjerda watershed (Table 1). Samples were collected with an aquatic net (300 μm mesh) and preserved in 70% ethyl alcohol. Identifications were performed with a binocular microscope using studies of Jansson (1986);

Table 2 List of species with their abbreviations

Infraorder	Family	Species name	Abbreviation
Nepomorpha	Nepidae	*Nepa cinerea* L.	Ncin
	Corixidae	*Micronecta scholtzi* F.	Msch
		Corixa affinis L.	Caff
		Sigara lateralis L.	Slat
		Sigara scripta R.	Sscr
		Sigara stagnalis stagnalis F.	Ssta
	Naucoridae	*Naucoris maculatus conspersus* S.	Ncon
	Notonectidae	*Anisops sardeus sardeus* H.	Asar
		Anisops debilis perplexus P.	Aper
		Notonecta maculata F.	Nmac
		Notonecta glauca glauca L.	Nglu
Gerromorpha	Hydrometridae	*Hydrometra stagnorum* L.	Hsta
	Mesoveliidae	*Mesovelia vittigera* H.	Mvit
	Gerridae	*Aquarius cinereus* P.	Aqci
		Gerris brasili P.	Gbra
		Gerris lacustris L.	Glac
		Gerris maculatus T.	Gmac
		Gerris thoracicus S.	Gth

Poisson (1957); Tamanini (1979). The taxonomy refers to the catalogues of Andersen (1971), Jansson (1995), Polhemus (1995a, b, c, d, e), Carapezza (1997) and Aukema et al. (2013).

A total of 18 species of aquatic and semi-aquatic Heteroptera were identified, of which 11 belonged to Nepomorpha and 7 to Gerromorpha. Both groups will be referred below as water bugs. Table 2 gives the list of species and the abbreviations used in multivariate analysis graphs.

2.2.3 Environmental parameters

Five environmental parameters were measured monthly during the sampling of water bugs: water pH (pH), salinity (S), conductivity (COND), total dissolved salt (TDS) and dissolved oxygen concentration (OXY). Measurements were collected in the field with portable equipment (WTW, MPP350). Air (AT) and water (WT) temperature were determined using a mercury thermometer sensitive to 0.1 °C. Flow speed (FS) was calculated as the time taken by a float (cork stopper) to cover a minimum distance of one meter. Turbidity (TUR) was measured in the laboratory using a turbidimeter (Hach model 2100A). Elevation was calculated using a GPS device (Garmin eTrex 10). Visual estimates were used to define the type of sediment in shallow water and riparian cover that characterized each site.

Other parameters, including BOD5, COD (Biological and Chemical Oxygen Demand), calcium, magnesium, chlorides, nitrates, amonium, and orthophosphate

concentration were measured only four times (once per season). These parameters were not included in the statistical analyses described here, except for orthophosphate (PO), which measures were repeated three times for each season.

3 Computations

In **ade4**, three-ways tables are handled as particular objects of class `ktab` (a `list` of `dataframes` sharing the same row names). A set of functions allows to handle these objects automatically. These functions perform operations like ktab creation, test, selection, concatenation, and tranposition.

The computation steps of the STATICO and COSTATIS methods are made easier by the use of two wrappers functions, `statico` and `costatis`.

3.1 Using the `statico` function

The first step is to load the **ade4** and **adegraphics** packages, and to set the factor corresponding to sampling dates (*i.e.* months) in chronological (*vs.* alphabetical) order. Note that here the parameter "`each=12`" corresponds to the number of sampling sites, and not to the number of months.

```
library(ade4)
library(adegraphics)
dat <- as.factor(rep(month.abb, each = 12))
datchron <- reorder(dat, rep(1:12, each = 12))
```

In a second step, the environmental parameters file is read and the `withinpca` function is used to do a within-group PCA with a special standardization. This procedure computes the residuals between data and the means by groups (here the means by month) and does a PCA on this table after a partial scaling (a global standardization, followed by a within-group standardization).

The `ktab.within` function is then used to create the `ktab` object `ktaEnv`. Starting from the table of (partially scaled) means by groups and the factor describing the groups (sampling dates), the `ktab.within` function creates the `ktab` object (list of dataframes) with the corresponding row weights.

```
env <- read.table("Env.txt", header = TRUE)
witEnv <- withinpca(env, datchron, scaling = "partial",
      scannf = FALSE, nf = 4)
ktaEnv <- ktab.within(witEnv, colnames = rep(1:12, 12))
```

The same procedure is applied to the water bug data: the file containing the water bug numbers is read, and log-transformed before using within-group PCA with partial scaling. This leads to the `ktab` object `ktaHet`.

```
het <- read.table("Het.txt", header = TRUE)
het <- log1p(het)
witHet <- withinpca(het, datchron, scaling = "partial",
```

```
       scannf = FALSE, nf = 4)
ktaHet <- ktab.within(witHet, colnames = rep(1:12, 12))
```

In the last step, the `statico` function takes the two `ktabs` as arguments and performs the STATICO analysis. `statico` is just a wrapper function, based on two other functions, `ktab.match2ktabs` and `pta`. The first one computes the cross-covariances between each pair of tables and builds a new `ktab` object containing the series of cross-covariance tables. The Partial Triadic Analysis is then done on this new `ktab` by the `pta` function.

```
stat1 <- statico(ktaEnv, ktaHet, scannf = FALSE)
```

The result `stat1` is therefore a PTA object.

3.2 Using the `costatis` function

The COSTATIS analysis can be computed with the `costatis` function on the same `ktab` objects. `costatis` is also a wrapper function that calls the `pta` function twice to do a Partial Triadic Analysis on each of the two `ktabs`. It then calls the `coinertia` function to do the Co-inertia analysis on the resulting Compromise tables.

```
cost1 <- costatis(ktaEnv, ktaHet, scannf = FALSE)
```

The result `cost1` is therefore a `coinertia` object.

4 Graphical displays

The graphical functions of the **adegraphics** package have been adapted to three-ways tables analysis methods. These functions implement particular (S4) methods for some generic functions (particularly `plot`) and many other functions are able to draw automatically collections of graphs corresponding to series of tables.

4.1 STATICO Interstructure and Compromise

The output of the `statico` function is a PTA object (class `pta`), and the generic `plot` function of the **adegraphics** package has a particular method for this class of objects. The figure shown here has been enhanced to add colors and avoid label superimpositions, and the **R** code to draw this figure is explained in "Appendix". Figure 3 shows the resulting graph for the `stat1` object. It is a compound figure, made of four elementary graphs.

The first graph (top left) corresponds to the Interstructure step. It shows the factor map of the 12 months in the eigenanalysis of the RV coefficients matrix. The two following graphs (top right and bottom left) show the factor maps of the analysis of the Compromise for environmental parameters and water bugs, respectively. The eigenvalues bar chart shows that the first two eigenvalues are distinctly higher than the following ones (73, 16, 5, 2% of total inertia for the first four axes). The last graph

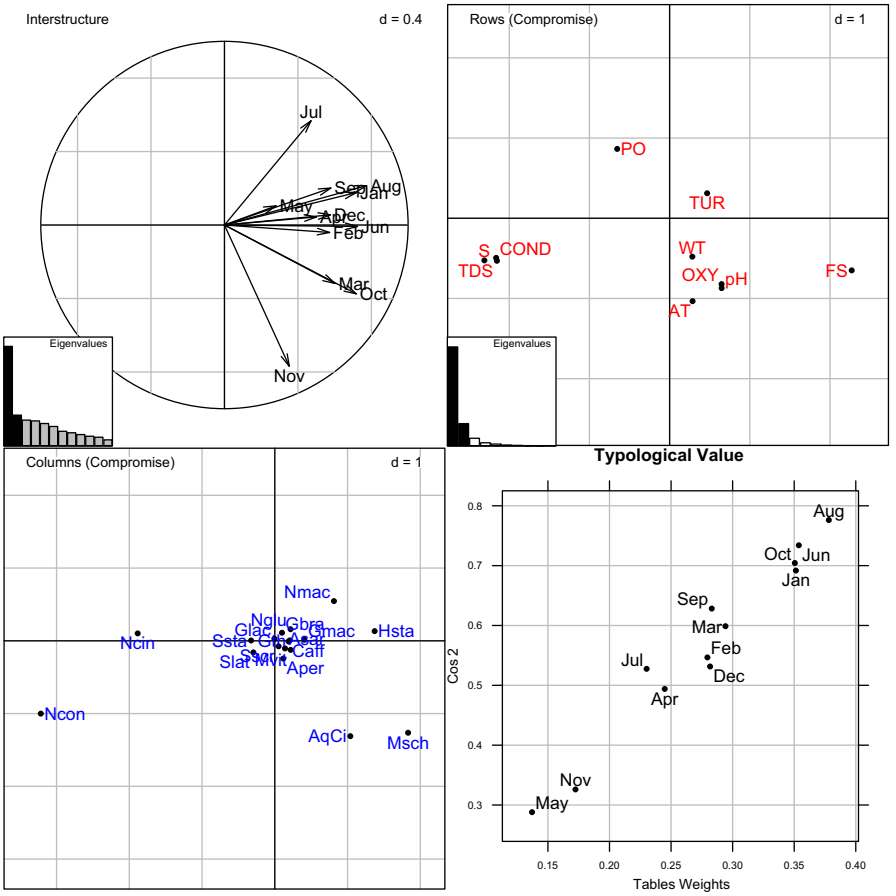

Fig. 3 General plot of the STATICO method (see text for explanations)

(bottom right) shows the typological value of the 12 months (*i.e.*, their influence in building the Compromise).

The months with the highest contribution to the Compromise are August and June. The months that have the lowest contribution are November and May.

On the factor map of environmental parameters in the Compromise analysis, the first axis (horizontal) describes a mineralization and salinity gradient. It is positively correlated with flow speed (FS, on the right), and negatively correlated with salinity, conductivity and total dissolved salts (S, COND, TDS, on the left). The second axis (vertical) is a pollution gradient, negatively correlated to air temperature (AT), pH and dissolved oxygen (OXY, down), and positively correlated to orthophosphates (PO, up).

On the factor map of water bugs, species are organized along these two gradients. Species of weakly mineralized water such as *Notonecta maculata* (Nmac) and *Hydrometra stagnorum* (Hsta) are on the right and species of highly mineralized water such as *Nepa cinerea* (Ncin) and *Naucoris maculatus conspersus* (Ncon) are on the left.

Species such as *Notonecta maculata* (Nmac) and *Hydrometra stagnorum* (Hsta) occur in slow to moderate speed streams, and accept high levels of pollution. Conversely, brackish taxa such as *Nepa cinerea* (Ncin) and *Naucoris maculatus conspersus* (Ncon) are associated with high values of salinity, conductivity and total dissolved salts, with slow streams and high nutrient levels linked to macrophyte coverage. Species *Micronecta scholtzi* (Msch) and *Aquarius cinereus* (Aqci) accommodate higher flow speeds, but are more sensitive to pollution and prefer high levels of dissolved oxygen and higher (more basic) pH.

The stable part of the species-environment relationship is therefore made of two components. The first one is the opposition between freshwater populations associated with clear, warm and slow-flowing waters, opposed to brackish populations characterized by higher salinity and conductivity of turbid and stagnant water. The second one is the opposition of water bugs species according to their resistance to anthropogenic pollution sources.

The next step (Intrastructure) shows that the gradients observed here can be noticed throughout the year, although some species characterize specific months.

4.2 STATICO Intrastructure of species and variables

In the Intrastructure step, the rows and columns of each table from the two series are projected in the analysis of the Compromise as supplementary elements. This is done in the same way as the projection of supplementary elements in a simple PCA (see for example Lebart et al. 1984).

Figure 4 shows the Intrastructure for the environmental parameters (red labels, columns of the environmental tables series) and for the water bugs (blue labels, columns of the species tables series). The **R** code to draw Fig. 4 is explained in "Appendix".

The mineralization and salinity gradient is obvious in Fig. 4, particularly for the summer months (June to October). The three parameters salinity, conductivity and total dissolved salts are grouped together in the left part of the graphs, and opposed to flow speed on the right. The pollution gradient (Oxygen and pH opposed to orthophosphates) is also visibly stronger from June to September.

In parallel, the opposition between freshwater and brackish water bugs is also clearly visible in Fig. 4. *Nepa cinerea* (Ncin) and *Naucoris maculatus conspersus* (Ncon) are on the left, and opposed to *Notonecta maculata* (Nmac) and *Hydrometra stagnorum* (Hsta) on the right. During the months where the pollution is the highest (August), the resistance of particular species (*Notonecta maculata* (Nmac) and *Hydrometra stagnorum* (Hsta)) is well visible. Conversely, *Aquarius cinereus* (Aqci) and *Micronecta scholtzi* (Msch) are more sensitive and prefer high values of pH, flow speed and oxygen concentration.

This relationship between environmental parameters and species preferences is the main feature of this dataset, and it is not surprising to find it on these figures. It can also be confirmed by the Intrastructure of sites, as the values of environmental parameters strongly depend on the position of sites along the stream.

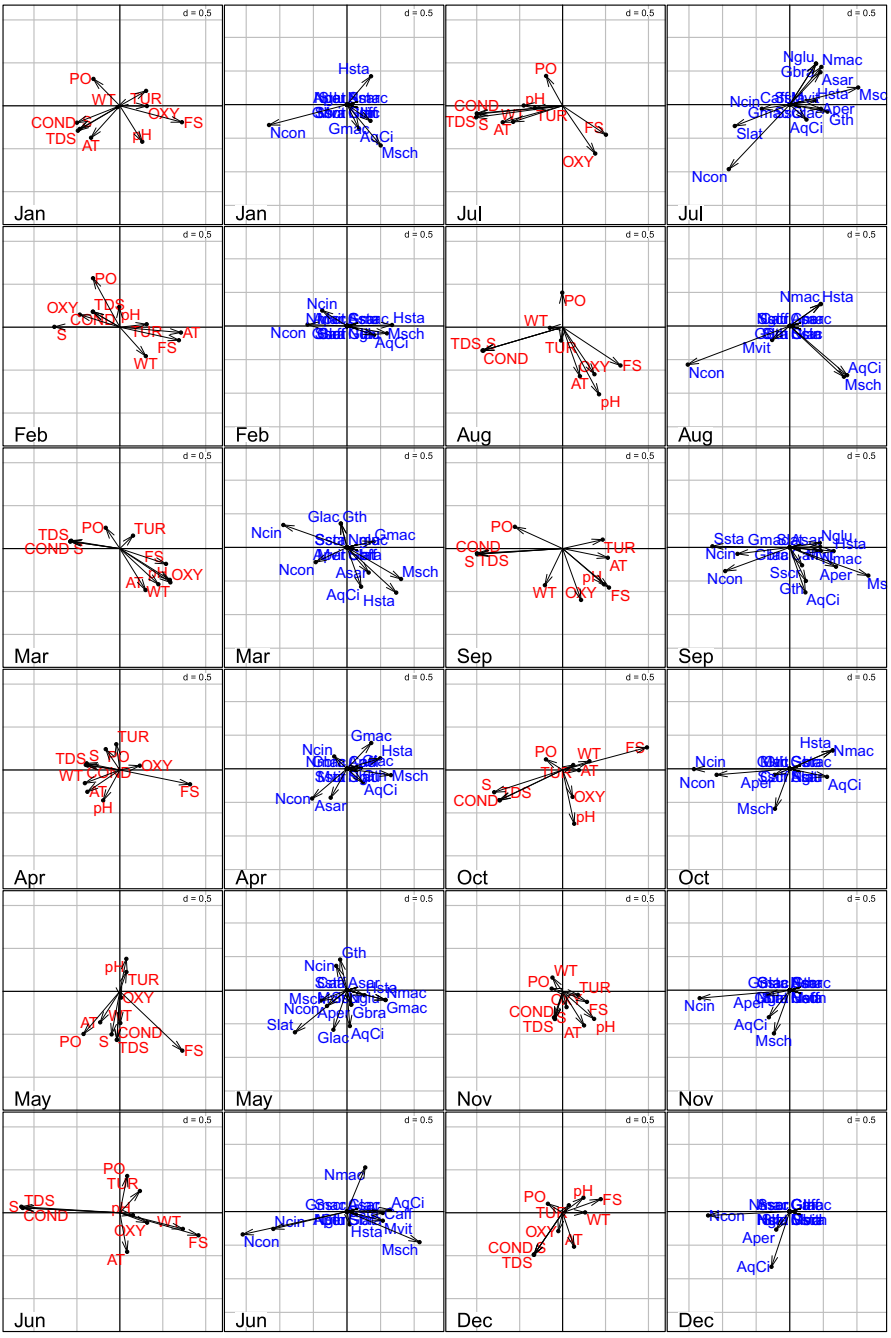

Fig. 4 Intrastructure plot of the STATICO method for environmental parameters (*red*, *left*) and water bugs (*blue*, *right*). The placement of elementary graphs allows to compare the pairs of graphs corresponding to each month (Color figure online)

4.3 STATICO Intrastructure of sites

Figure 5 shows the Intrastructure for the sampling sites of the environmental parameters (red labels) and of water bugs (blue labels). The **R** code to draw Fig. 5 is explained in "Appendix". The variations along the upstream–downstream gradient is very clear, and confirms the influence of environmental parameters on water bugs distribution (see Fig. 4).

From the point of view of environmental parameters (red labels, upper part of the figure) sites are roughly ordered along the upstream-downstream gradient from right to left. This is in accordance with the mineralization and salinity gradient observed previously. In late spring (May) and summer (June, July, and August), site 5 and 6 move upward. This corresponds to the increased pollution at these sites, linked to an increase in water and air temperature, an absence of precipitation, and a decrease of flow speed. The low values of disolved oxygen (OXY) at these sites (0.3 mg/l) are consistent with this interpretation. In late summer and winter, only site 5 stays in the upper part of the graph, showing that pollution is highest at this site even in winter.

From the point of view of water bugs (blue labels, lower part of the figure), site distribution also has the same structure across months, along the upstream–downstream gradient. Sites 5 and 6 move to the upper right position from May to October because a group of species *Notonecta maculata*, *Hydrometra stagnorum*, *Gerris maculatus* (Nmac, Hsta, Gmac) was sampled in large numbers during summer. This feature is linked to the fact that these species are more resistant to pollution, while others are more sensitive.

4.4 COSTATIS Co-inertia results

As explained in the Statistical Methods section (Sect. 2.1), COSTATIS is the Co-inertia analysis of the two Compromises obtained by the Partial Triadic Analysis of each of the two series of tables separately (one PTA for environmental parameters and one for water bugs).

Figure 6 is the plot of this Co-inertia analysis. It can be drawn using the generic `plot` function for `coinertia` objects, but the figure shown here has been enhanced to add colors and avoid label superimpositions. The **R** code to draw this figure is explained in "Appendix".

The main graph (top right) shows 12 arrows, representing the 12 sites. The tip of the arrow is the site seen from the point of view of water bugs, and the other end of the arrow (black bullet) is the site seen from the point of view of environmental parameters. The length of these arrows is therefore a measure of the discrepancy between the two data tables. Strong cross-covariances between the variables of the two tables mean that the sites are similar. In this case, the two points are near on the graph and the corresponding arrow is short.

The two correlation circles on the left of the figure show the projection of unconstrained axes in the Co-inertia factor map. Here, they correspond to the axes of the two separate PTA. They show that Co-inertia analysis axes are closely related to the axes

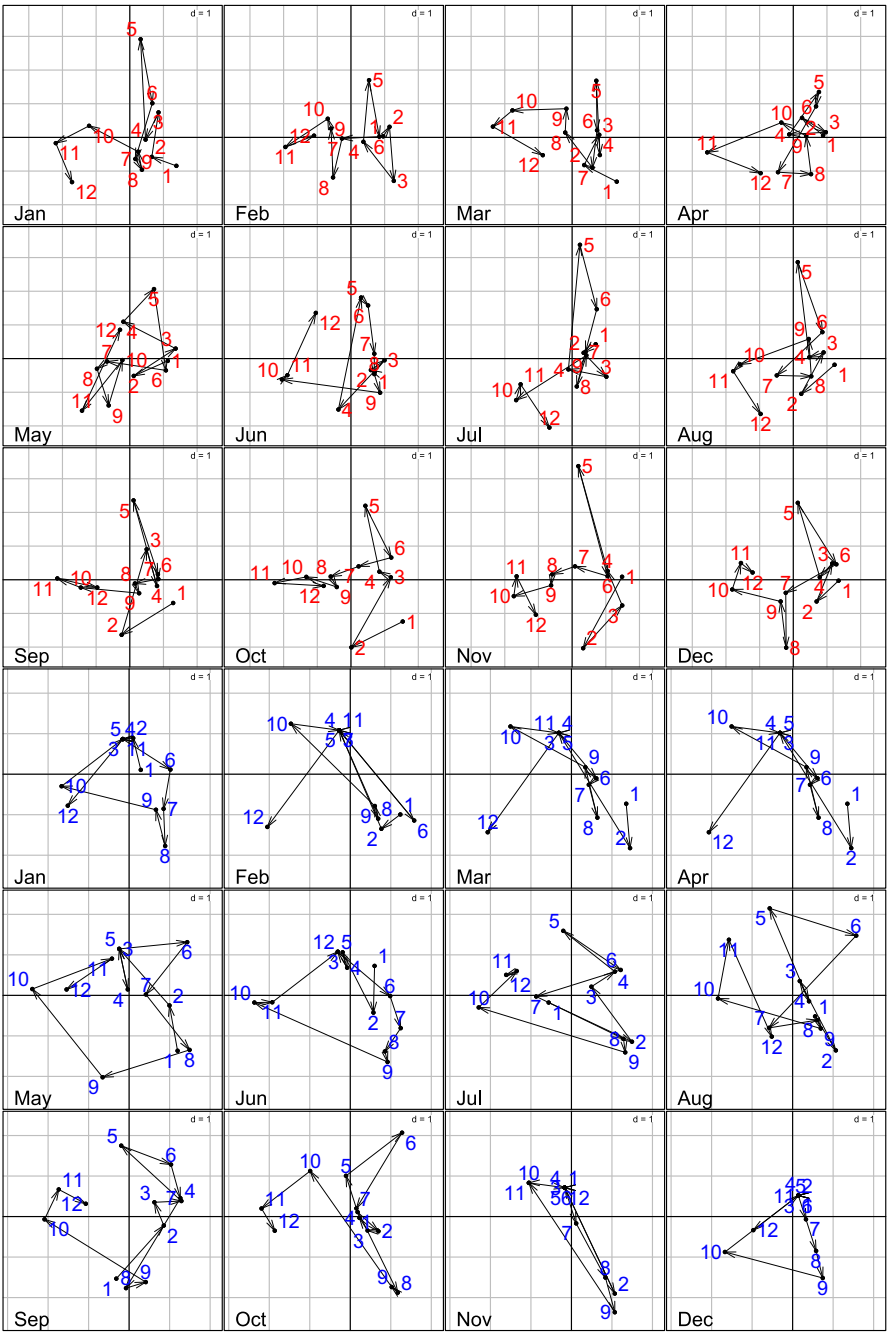

Fig. 5 Intrastructure plot of the STATICO method for the sampling sites of the environmental parameters (*top*, *red labels*) and water bugs (*bottom*, *blue labels*) (Color figure online)

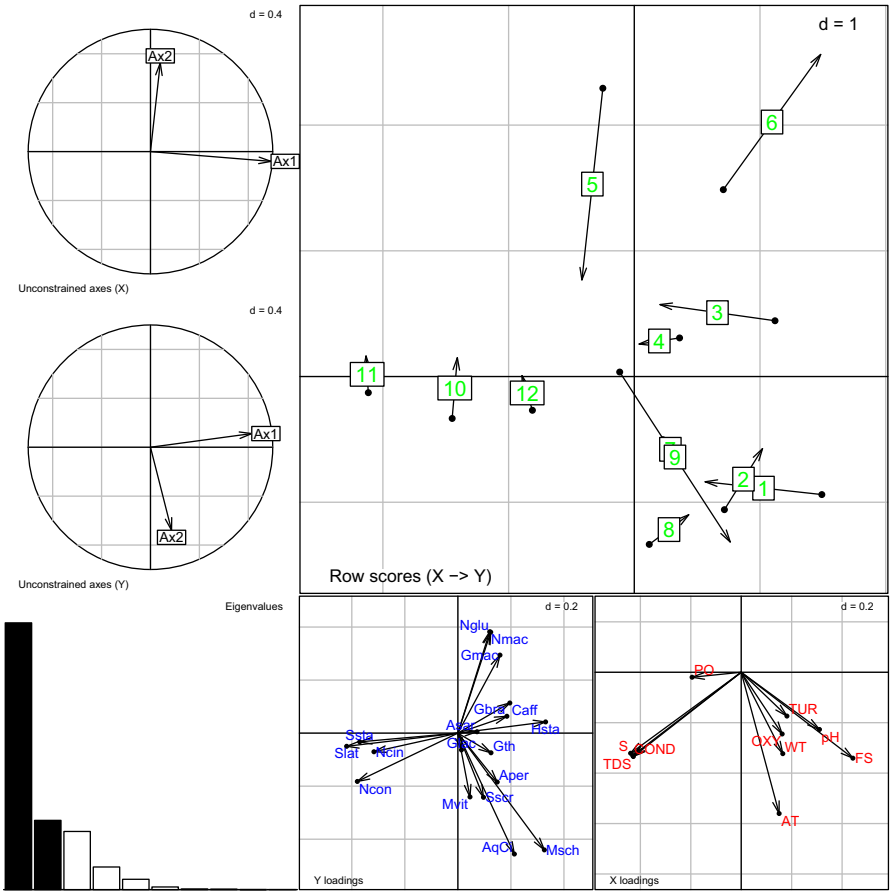

Fig. 6 General plot of the COSTATIS method (see text for explanations)

of the separate analyses. For environmental parameters (X, top) both axes of the PTA are equivalent to the axes of the COSTATIS analysis. For water bugs (Y, bottom), axis 1 is equivalent, while axis 2 is merely the opposite of the corresponding COSTATIS axis.

The lower left graph is the eigenvalues bar chart, showing that the first eigenvalue is much more important than the following ones (61, 16, 13, 5% of total inertia for the first four axes). The two graphs in the lower part of the figure are the graphs of water bugs and of environmental parameters.

Downstream sites (10, 11, 12) are on the left, characterized by high mineralization and high abundances of *Sigara (Vermicorixa) lateralis*, *Sigara stagnalis stagnalis*, *Naucoris maculatus conspersus* and *Nepa cinerea* (Slat, Ssta, Ncon Ncin). These four species are also located on the left part of the water bugs graph.

Upstream sites (1–9) are located in the lower-right part of the graph, with the exception of sites 5 and 6, which are located in the upper right. Some species like *Micronecta scholtzi* (Msch) and *Aquarius cinereus* (Aqci) are more abundant in these upstream

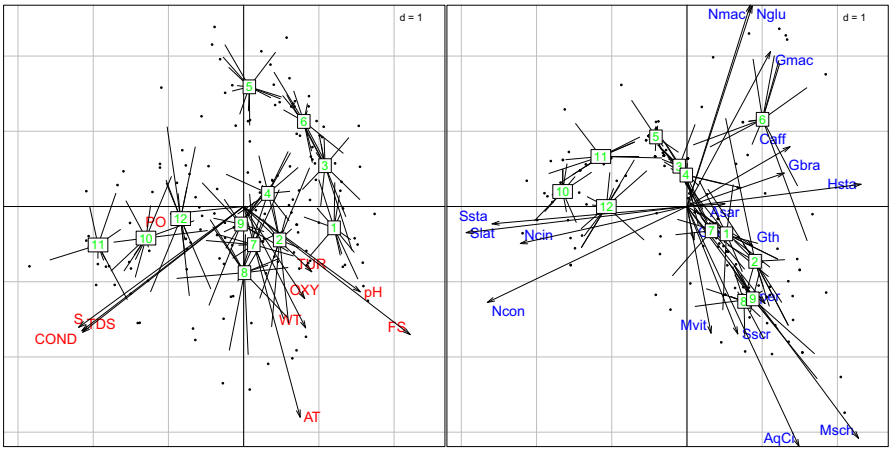

Fig. 7 Intrastructure plot of the COSTATIS method: projection of the rows (sampling sites, *green labels*) of the two series of tables, superimposed with environmental parameters (*left*, *red labels*) and water bugs (*right*, *blue labels*) (Color figure online)

sites. Species *Gerris maculatus* (Gmac), *Notonecta glauca* (Nglu) and *Notonecta maculata* (Nmac) are more resistant to pollution and are mainly found in site 6. The case of site 5 (Wadi Kasseb) is exceptional by the total absence of aquatic Heteroptera fauna, because of very low values of dissolved oxygen (less than 0.3 mg/l), with an increased rate of orthoposphate (PO). This has adverse effects on wildlife and particularly on water bugs, and it is linked to the waste waters of a dairy plant located just upstream site 5.

The two orthogonal gradients of environmental parameters that were observed on Fig. 3 in the STATICO analysis are also visible here, with the oppositon between variables TDS, COND, S, and FS (salinity gradient) and the opposition between Oxygen and orthophosphates (pollution gradient).

4.5 COSTATIS Intrastructure of sites

Figure 7 shows the Intrastructure of the COSTATIS analysis, and it is very easy to interpret. The left part of the figure is the superimposition of environmental parameters (red labels) and sites (1–12, green labels). The right part is the superimposition of water bug species (blue labels) and sites (1–12, green labels). This figure is a good summary of the upstream-downstream salinity gradient and of the pollution effect. It shows the impact of environmental parameters on water bug species distribution along the 12 sampling sites. The **R** code to draw Fig. 7 is explained in "Appendix".

5 Discussion

Both data analysis methods show an upstream-downstream gradient of mineralization on axis 1, characterized by the salinity, conductivity and total dissolved salts. They

also show a gradient of pollution on axis 2, mainly characterized by oxygen and orthophosphates concentrations, opposing sites 5 and 6 to the other ones.

Medjerda watershed is characterized by a relatively strong overall mineralization, speed flow and clear waters. These results are in agreement with those of Rodier et al. (1981), revealing high concentrations of dissolved materials in the Medjerda watershed. They were the strongest concentrations observed in the world with Boumaïza (1984), who indicated that the mineralization was higher in tributaries of the south bank of the Medjerda watershed compared to the rivers of northern Tunisia. In addition, assessment of Medjerda river water quality according to the FAO standard for use in irrigation indicated possible problems in terms of sodium and chloride toxicity (Numaan 2011).

The spatial structure of water bugs shows a species distribution dominated by six species upstream (ST1, ST2, ST3, ST4, ST6 and ST7), namely, *Aquarius cinereus* (Aqci), *Micronecta scholtzi* (Msch), *Corixa affinis* (Caff), *Notonecta glauca glauca* (Nglu), *Gerris maculatus* (Gmac) and *Gerris brasili* (Gbra). Downstream sites (ST10, ST11 and ST12) are mainly characterized by *Nepa cinerea* (Ncin), *Sigara stagnalis stagnalis* (Ssta), *Sigara lateralis* (Slat) and *Naucoris conspersus maculatus* (Ncon). The other species (*Sigara scripta* (Sscr), *Anisops debilis perplexus* (Aper), *Anisops sardea sardius* (Asar), *Notonecta maculata* (Nmac), *Hydrometra stagnorum* (Hsta), *Mesovelia vittigera* (Mvit), *Gerris lacustris* (Glac), *Gerris thoracicus* (Gth) may form a transitional group, distributed from the headwaters to the lower sections of the Medjerda basin with stagnant or low-flow environments. Site ST5 is characterized by the lowest dissolved oxygen value and the highest chemical pollution, linked to the presence of a dairy plant (Abidi et al. 2011, 2015).

The difference in the environmental characteristics between upstream and downstream is partly due to the construction of several irrigation dams that increase trophic resources downstream. Thus, it favors the presence of species such as *Nepa cinerea* (Ncin), which was common in pools with rich vegetation (Garcia-Aviles et al. 1996).

Temporal variation shows that water bugs community structure is correlated with abiotic conditions changing through time. Not surprisingly, most of the species are sampled during spring and summer when air and water temperatures are high. The Medjerda valley is characterized by an important flow regime from December to March driving occasionally large floods (Zahar et al. 2008). This natural event has an impact on faunistic richness.

From a more methodological point of view, both methods (STATICO and COSTATIS) reveal the same structures in this complex data set. STATICO is a three-ways analysis of the series of cross-covariance tables between each pair of data tables. It provides a slightly more detailed view of data structures, at the expense of a somewhat more complex interpretation. Conversely, COSTATIS is a simple Co-inertia analysis of two Compromises resulting from the separate three-ways analysis of the two series of tables. It provides a more synthetic view of structures and simpler interpretation.

The **ade4** package provides simple procedures to perform these analyses, with adapted data structures and functions for three-ways tables handling. The **adegraphics** package offers compound graphs for both methods and automatic graphic collection procedures that make much simpler the interpretation of numerical outputs.

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Appendices

General plot of the STATICO method (Fig. 3)

This figure can be drawn with the generic `plot` function, but the version given here is enhanced to add colors and avoid label superimpositions. Note that this figure should be drawn in a square window to keep an appropriate height/width ratio.

```
g11 <- s.corcircle(stat1$RV.coo, psub = list(text = "Interstructure",
  position = "topleft"), pbackground.box = FALSE, plabels.cex = 1.25,
  plabels.boxes.draw = FALSE, plot = FALSE)
g12 <- plotEig(stat1$RV.eig, nf = 1:length(stat1$RV.eig),
  psub.text = "Eigenvalues", pbackground.box = TRUE, plot = FALSE)
g1 <- insert(g12, g11, posi = "bottomleft", plot = FALSE,
  ratio = 0.25, inset = 0)
g2 <- s.label(stat1$co, psub = list(text = "Columns (Compromise)",
  position = "topleft"), plabels = list(cex = 1.25, col="blue",
  optim = TRUE), plot = FALSE)
g31 <- s.label(stat1$li, psub = list(text = "Rows (Compromise)",
  position = "topleft"), plabels = list(cex = 1.25, col="red",
  optim = TRUE), plot = FALSE)
g32 <- plotEig(stat1$eig, nf = 1:stat1$nf, psub.text = "Eigenvalues",
  pbackground.box = TRUE, plot = FALSE)
g3 <- insert(g32, g31, posi = "bottomleft", plot = FALSE,
  ratio = 0.25, inset = 0)
g4 <- s.label(matrix(c(stat1$tabw, stat1$cos2), nrow =
  length(stat1$tabw), ncol = 2, dimnames = list(rownames(stat1$RV))),
  porigin.include = FALSE, paxes = list(aspectratio = "fill",
  draw = TRUE), main = "Typological Value", xlab = "Tables Weights",
  ylab = "Cos 2", plabels = list(cex = 1.25, optim = TRUE),
  plot = FALSE)
gtot <- ADEgS(list(g1, g2, g3, g4),
  layout = matrix(c(1, 2, 3, 4), 2, 2))
```

Graph `g11` is the correlation circle of the Interstructure (top left). The eigenvalues bar chart `g12` of the Interstructure analysis is drawn with the `plotEig` function and inserted in the correlation circle graph with the `insert` function to obtain graph `g1`.

Graph `g2` is the factor map of Compromise columns (bottom-left). It is drawn with the `s.label` function and label color is set to `blue`. The `plabels.optim`

parameter is set to TRUE, which means that labels are arranged to minimize superimpositions.

Graph g31 is the factor map of Compromise rows (top-right). It is also drawn with the s.label function and label color is set to red. The eigenvalues bar chart g32 of the Compromise analysis is drawn with the plotEig function and inserted in graph 31 with the insert function to obtain graph g3.

Graph g4 is the plot of the "typological value" (squared cosines vs. weights) of the tables. It is drawn with the s.label function.

The four graphs are finally grouped using the ADEgS function to get the final Fig. gtot.

STATICO Intrastructure for environmental parameters and water bugs (Fig. 4)

This figure uses the facets argument to draw automatically the graphs corresponding to the environmental parameters and species at each date (12 months). The height/width ratio of the window in which this figure is drawn should be set to 1.5 to keep appropriate scales.

```
slE <- s.label(stat1$Tli, facets = stat1$TL[, 1],
  labels = stat1$TL[, 2], psub.cex = 2, plabel=list(col = "red",
  cex=1.5, optim=TRUE), plot=FALSE)
saE <- s.arrow(stat1$Tli, facets = stat1$TL[, 1], psub.cex = 0,
  plabels.cex=0, plines.lwd=0.5, plot=FALSE)
sE <- superpose(slE, saE)
slH <- s.label(stat1$Tco, facets = stat1$TC[, 1],
  labels = stat1$TC[, 2], psub.cex = 2, plabel=list(col = "blue",
  cex=1.5, optim=TRUE), plot=FALSE)
saH <- s.arrow(stat1$Tco, facets = stat1$TC[, 1], psub.cex = 0,
  plabel.cex=0, plines.lwd=0.5, plot=FALSE)
sH <- superpose(slH, saH)
sE1 <- sE[1:6]
sE2 <- sE[7:12]
sH1 <- sH[1:6]
sH2 <- sH[7:12]
sE1@positions <- layout2position(c(6,1))
sE2@positions <- layout2position(c(6,1))
sH1@positions <- layout2position(c(6,1))
sH2@positions <- layout2position(c(6,1))
sEH1 <- ADEgS(list(sE1, sH1), layout=c(1,2), plot=FALSE)
sEH2 <- ADEgS(list(sE2, sH2), layout=c(1,2), plot=FALSE)
sEH <- ADEgS(list(sEH1, sEH2), layout=c(1,2))
```

Four graph collections are drawn with the s.label and s.arrow function, using the facets argument: slE (labels) and saE (arrows) for environmental parameters (red labels), and slH (labels) and saH (arrows) for water bugs (blue labels).

Each collection is made of the 12 graphs corresponding to the 12 months with the facets argument and the TL or TC elements of the stat1 object. These elements

contain factors defining to which month belongs each environmental parameter or each water bug species.

The collections of labels and arrows graphs are superimposed with the `superpose` function. They are then split in two (months January to June, and months July to December), and the positions of the elementary graphs corresponding to the 6 months are rearranged to place side by side the environmental parameters graph and the water bugs graph of each pair.

This rearrangement of elementary graph positions is done with the `layout2position` function. It allows an easier comparison of species and environmental parameters graphs month by month.

Both collections of graphs are grouped again using the `ADEgS` function and plotted side by side.

STATICO Intrastructure for the sampling sites (Fig. 5)

This figure also uses the `facets` argument to draw automatically the graphs corresponding to the sampling sites of the environmental parameters table and of the species data table at each date (12 months). The height/width ratio of the window in which this figure is drawn should be set to 1.5 to keep appropriate scales.

```
st1 <- s.traject(stat1$supIX, facets=stat1$supTI[,1], plabels.cex=0,
  plot=FALSE, psub.cex=0, plines.lwd=0.5)
sla1 <- s.label(stat1$supIX, facets=stat1$supTI[,1], plot=FALSE,
  psub.cex=2, labels=stat1$supTI[,2], plabels=list(cex=2, col="red",
  optim=TRUE))
s1 <- superpose(st1, sla1)
st2 <- s.traject(stat1$supIY, facets=stat1$supTI[,1], plabels.cex=0,
  plot=FALSE, psub.cex=0, plines.lwd=0.5)
sla2 <- s.label(stat1$supIY, facets=stat1$supTI[,1], plot=FALSE,
  psub.cex=2, labels=stat1$supTI[,2], plabels=list(cex=2, col="blue",
  optim=TRUE))
s2 <- superpose(st2, sla2)
ADEgS(list(s1,s2), layout = c(2,1))
```

Intrastructure plot of the STATICO method for the sampling sites of the environmental parameters (top, red labels) and water bugs (bottom, blue labels).

In this figure, the `facets` argument of the `s.traject` and `s.label` functions is used to draw automatically collections of graphs. In these collections, each elementary graph corresponds to one table (*i.e.*, one month). The selection of the rows that go into each graph is done with the `stat1$supTI` factor that is built during analysis computations.

The first collection of graphs (`st1`) is trajectory lines that links the 12 sites of the environmental parameters tables in the upstream-downstream order. The second collection (`sla1`) draws the site labels (1–12, in red). Both collections are superimposed with the `superpose` function, resulting in graph `s1`.

The same procedure is used for the 12 sites of the water bugs tables (with blue labels), resulting in graph s2. Graphs s1 and s2 are placed one under the other and plotted with function ADEgS.

General plot of the COSTATIS method (Fig. 6)

This figure can be drawn with the generic plot function, but the version given here is enhanced to add colors and avoid label superimpositions. This figure should be drawn in a square window to keep an appropriate height/width ratio.

```
g1 <- s.corcircle(cost1$aX, psub.text = "Unconstrained axes (X)",
  pbackground.box = FALSE, plabels.cex = 1.25, plot = FALSE)
g2 <- s.corcircle(cost1$aY, psub.text = "Unconstrained axes (Y)",
  pbackground.box = FALSE, plabels.cex = 1.25, plot = FALSE)
g3 <- plotEig(cost1$eig, nf = 1:cost1$nf, psub.text = "Eigenvalues",
  plot = FALSE)
g4 <- s.match(cost1$mX, cost1$mY, psub.text = "Row scores (X -> Y)",
  plabels = list(cex = 1.25, col="green"), plot = FALSE)
g51 <- s.arrow(cost1$l1, plabels.cex = 0, plot = FALSE)
g52 <- s.label(cost1$l1, psub.text = "Y loadings", plabels =
  list(cex = 1.25, col="blue", optim = TRUE), plot = FALSE)
g61 <- s.arrow(cost1$c1, plabels.cex = 0, plot = FALSE)
g62 <- s.label(cost1$c1, psub.text = "X loadings", plabels =
  list(cex = 1.25, col="red", optim = TRUE), plot = FALSE)
gtot <- ADEgS(list(g1, g2, g3, g4, g51 + g52, g61 + g62),
  layout = matrix(c(1, 2, 3, 4, 4, 5, 4, 4, 6), 3, 3))
```

There are six elementary graphs that correspond to several elements of the COSTATIS analysis numerical outputs.

The two correlation circles on the left of the figure show the projection of unconstrained axes in the Co-inertia factor map. They correspond here to the axes of the two separate PTA. They are drawn with the s.corcircle function and stored in objects g1 and g2. The eigenvalues bar chart is drawn with the plotEig function, giving object g3.

The main graph is graph g4. It is a special graph, drawn with the s.match function. This function takes two sets of coordinates for the same series of points and draws an arrow between each pair of coordinates. Here, the two series of coordinates are cost1$mX, the coordinates of the sites in the environmental parameters tables and cost1$mY, the coordinates of sites in the species tables. The twelve arrows are numbered 1–12 and correspond to the 12 sites (green labels).

The two graphs in the lower part of the figure are the graphs of water bugs and of environmental parameters. Each one is drawn with the s.arrow and s.label function resulting in objects g51 and g52 (water bug species, blue labels) and g61 and g62 (environmental parameters, red labels). The two graphs of each pair are superimposed with the + operator.

The final figure gtot is obtained by joining the six elementary graphs with the ADEgS function and a fixed layout that allocates more space to the main graph g4.

COSTATIS Intrastructure plot (Fig. 7)

This is a synthetic figure, showing the superimposition of the rows (sampling sites: 1–12) and columns (environmental parameters: red labels and water bugs: blue labels) of both series of tables. The height/width ratio of the window in which this figure is drawn should be set to 0.5 to keep appropriate scales.

```
xlt <- c(min(cost1$supIX[,1], cost1$c1[,1]*5, cost1$supIY[,1],
  cost1$l1[,1]*7), max(cost1$supIX[,1], cost1$c1[,1]*5,
  cost1$supIY[,1], cost1$l1[,1]*7))
ylt <- c(min(cost1$supIX[,2], cost1$c1[,2]*5, cost1$supIY[,2],
  cost1$l1[,2]*7), max(cost1$supIX[,2], cost1$c1[,2]*5,
  cost1$supIY[,2], cost1$l1[,2]*7))
lim1 <- c(min(xlt, ylt), max(xlt, ylt))
sl1 <- s.label(cost1$c1*5, xlim=lim1, ylim=lim1, label =
  row.names(cost1$c1), plabels = list(cex = 1.5, col = "red",
  optim = TRUE), ppoints.cex=0, plot = FALSE)
sa1 <- s.arrow(cost1$c1*5, xlim=lim1, ylim=lim1, label =
  row.names(cost1$c1), plabels.cex = 0, plabels.boxes.draw = FALSE,
  psub.cex = 0, plines.lwd = 0.5, plot = FALSE)
sc1 <- s.class(cost1$supIX, xlim=lim1, ylim=lim1,
  fac = ktaHet$TC[,2], ellipseSize = 0, starSize = 0.7,
  plabels = list(cex=1.25, col = "green"), ppoints.cex = .5,
  plines.lwd = 0.5, plot = FALSE)
ss1 <- superpose(superpose(sl1, sa1, plot = FALSE), sc1,
  plot = FALSE)
sl2 <- s.label(cost1$l1*7, xlim=lim1, ylim=lim1, label =
  row.names(cost1$li), plabels = list(cex = 1.5, col = "blue",
  optim = TRUE), ppoints.cex=0, plot = FALSE)
sa2 <- s.arrow(cost1$l1*7, xlim=lim1, ylim=lim1, label =
  row.names(cost1$li), plabels.cex = 0, psub.cex = 0,
  plines.lwd = 0.5, plot = FALSE)
sc2 <- s.class(cost1$supIY, xlim=lim1, ylim=lim1,
  fac = ktaHet$TC[,2], ellipseSize = 0, starSize = 0.7,
  plabels = list(cex=1.25, col = "green"), ppoints.cex = .5,
  plines.lwd = 0.5, plot = FALSE)
ss2 <- superpose(superpose(sl2, sa2, plot = FALSE), sc2,
  plot = FALSE)
st1 <- ADEgS(list(ss1, ss2), layout = c(1,2))
```

This figure is composed of two graphs: the environmental parameters graph (left) and the water bugs graph (right). The limits of the four (scaled) coordinate vectors, `cost1$supIX`, `cost1$c1`, `cost1$supIY` and `cost1$l1` are first computed to set the same limits for all the graphs.

The environmental parameters graph is the superimposition of three elementary graphs: `sl1` (`s.label` function, red labels), `sa1` (`s.arrow` function) for parameters, and `sc1` (`s.class` function, green labels grouped by site) for sampling sites.

These three graphs are superimposed with the `superpose` function to get the first part of the Fig. (`ss1`).

The water bugs graph is also the superimposition of three elementary graphs: `sl2` (`s.label` function, blue labels), `sa2` (`s.arrow` function) for Heteroptera species, and `sc2` (`s.class` function, green labels grouped by site) for sampling sites. These three graphs are superimposed with the `superpose` function, leading to the second graph `ss2`.

Graphs `ss1` and `ss2` are grouped side by side with the `ADEgS` function to get the complete Fig. `st1`.

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