Plausible Bioindicators of Biological Nitrogen Removal Process in WWTPs.
Application of Multivariate Predictive Models

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Introduction

The optimization of wastewater treatment involves the search of new tools for the control of the process. Nowadays, restrictions concerning the discharge of certain pollutants, such as nitrogen and phosphorus are imposed, so new configuration of treatment plants have been proposed (fig. 1).

Figure 1. (a) Eutrophication process. (b) Configuration of WWTP.

Advances in the knowledge of dynamics of the protists and metazoans populations are necessary for the proposal of new biological control tools in activated sludge. Many studies have attempted to relate these organisms with physicochemical and operational variables of wastewater treatment plants (WWTPs) in order to reveal possible bioindicators [1]. However, these studies have been mainly descriptive and/or exploratory and environmental interpretation has not been included in them. Therefore, the aim of this study was the environmental ordination of the relationships between biological variables (protists and metazoans) and physicochemical variables (nitrogen compounds), for the proposal of bioindicators of the nitrogen removal process in WWTPs.

Material & Methods

Sampling: Samples from activated sludge (n=140), influent (n=420) and treated effluent (n=140) were collected every fifteen days during a year from six bioreactors belonging to four different WWTPs located in Spain (QB, CX, DN and CT).

Biological processing: Density of protists and metazoans was obtained by direct counting (six replicates of 25 μL) and different staining procedures, using phase contrast microscopy (fig. 2). To avoid background noise, only those biological variables that showed a frequency of appearance in samplings higher than 20% were selected for the multivariate analysis.
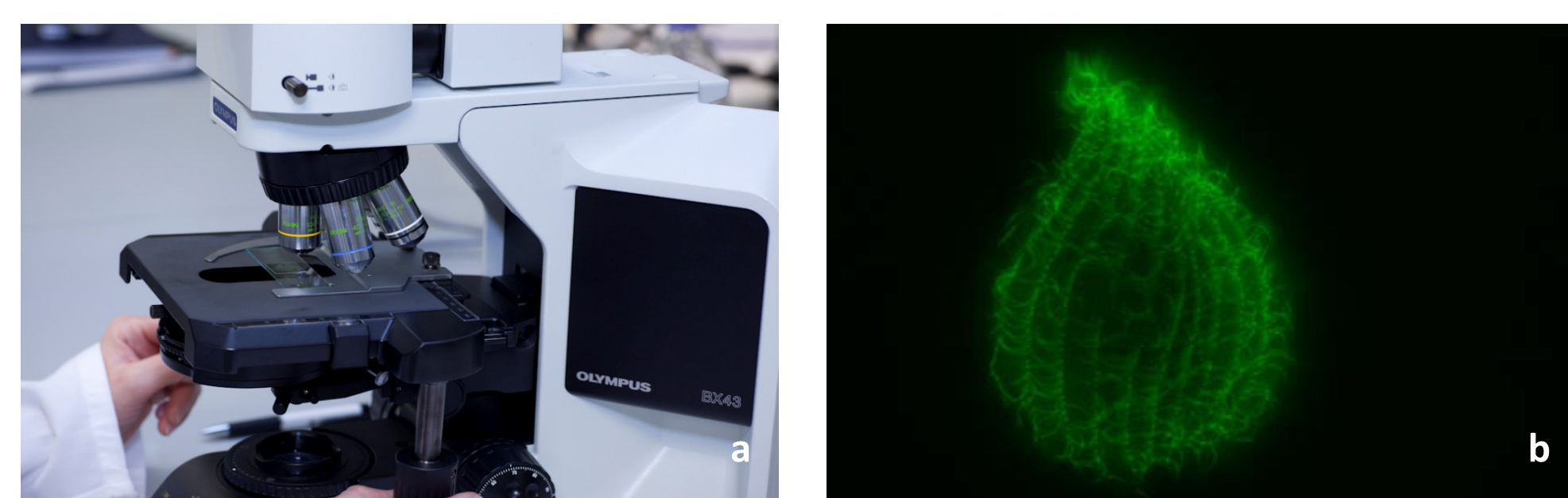

Figure 2. Biological processing. (a) Microscopic counting. (c) Flutax staining.

Multivariate analysis: Non-metric multidimensional scaling (nMDS), principal coordinates analysis (PCoA) and hierarchical cluster analysis (cluster) were used to evaluate the spatial-temporal variability of biological variables (protists and metazoans) by examining the relative distances among samples and variables in the ordination (abundance log-transformed data; Bray-Curtis similarity; group-average linking). To assess the plausible bioindicators of biological nitrogen removal process in WWTPs, we use a double approach: lineal and unimodal techniques. We carried out distance-based linear models (DISTLM), using parsimonious methods (e.g. BIC, AIC$_C$), and unimodal models employing canonical correspondence analysis (CCA). Nitrogen compounds were log-transformed and normalized to eliminate their physical units, prior to multivariate data analyses (euclidean similarity). Distance-based redundancy analysis (dbRDA) was used to visualize the DISTLM. All multivariate analyses were performed with PRIMER v7 [2] with PERMANOVA+ [3] and CANOCO for Windows 4.5 [4].

Results & Discussion

Fifty-one species of protists and six of metazoans were identified in the active sludge samples investigated. A total of thirty-two species were selected for the multivariate analysis (fig. 3).
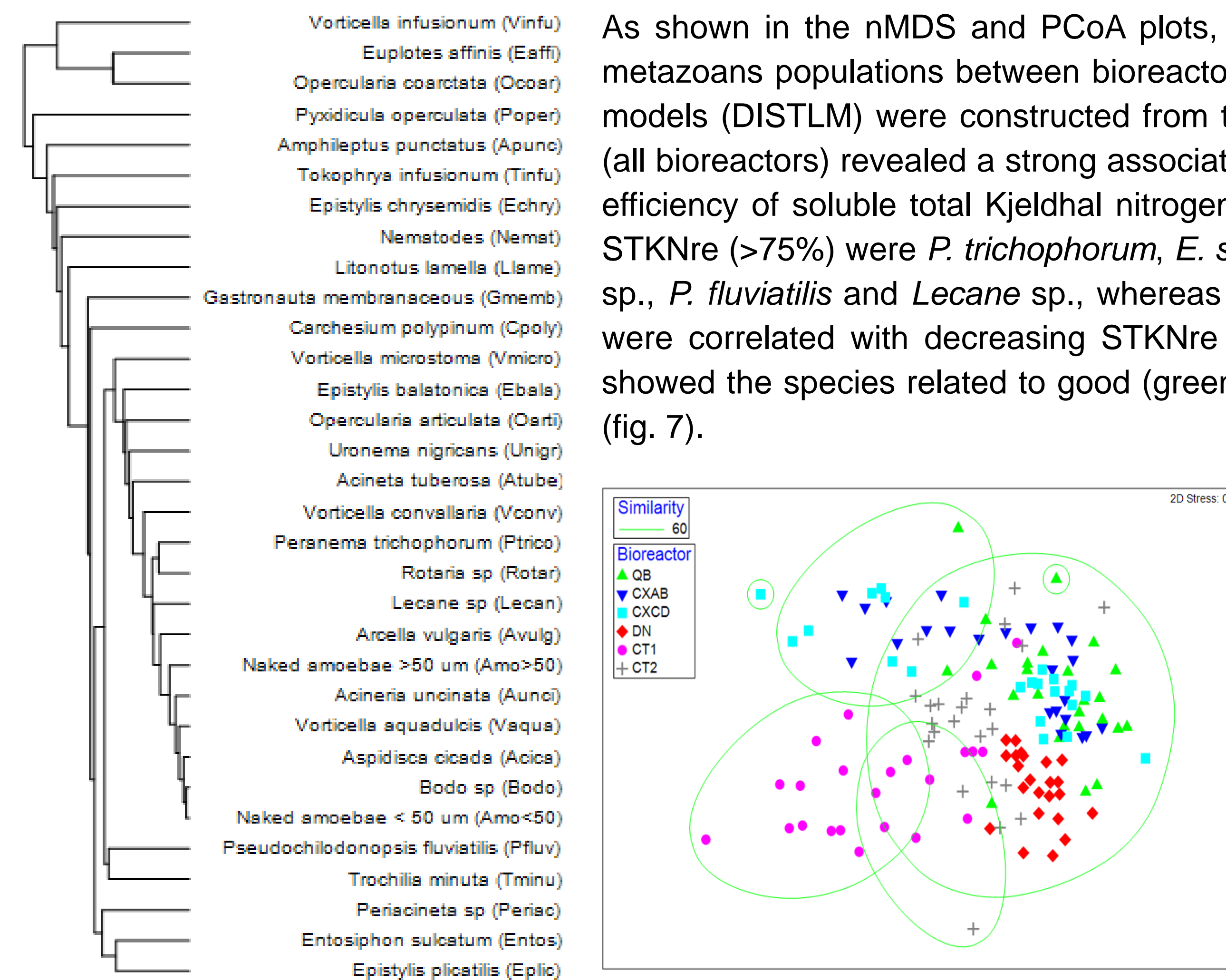

Figure 3. Species clustering ordering

As shown in the nMDS and PCoA plots, the results revealed some differences in protists and metazoans populations between bioreactors (fig. 4) and seasons (fig. 5). A total of six predictive models (DISTLM) were constructed from the six bioreactors. The dbRDA plot of the total matrix (all bioreactors) revealed a strong association of several protists and metazoans with the removal efficiency of soluble total Kjeldhal nitrogen (STKNre) (fig. 6). Species correlated with increasing STKNre (>75%) were *P. trichophorum*, *E. sulcatum*, *A. tuberosa*, *O. articulata*, *E. plicatilis*, *Rotaria* sp., *P. fluviatilis* and *Lecane* sp., whereas that *A. cicada*, *V. aquadulcis* and *O. coarctata* (fig. 7) were correlated with decreasing STKNre (<75%). On the other hand, the CCA ordination plot showed the species related to good (green color) and poor (black color) nitrification performance (fig. 7).
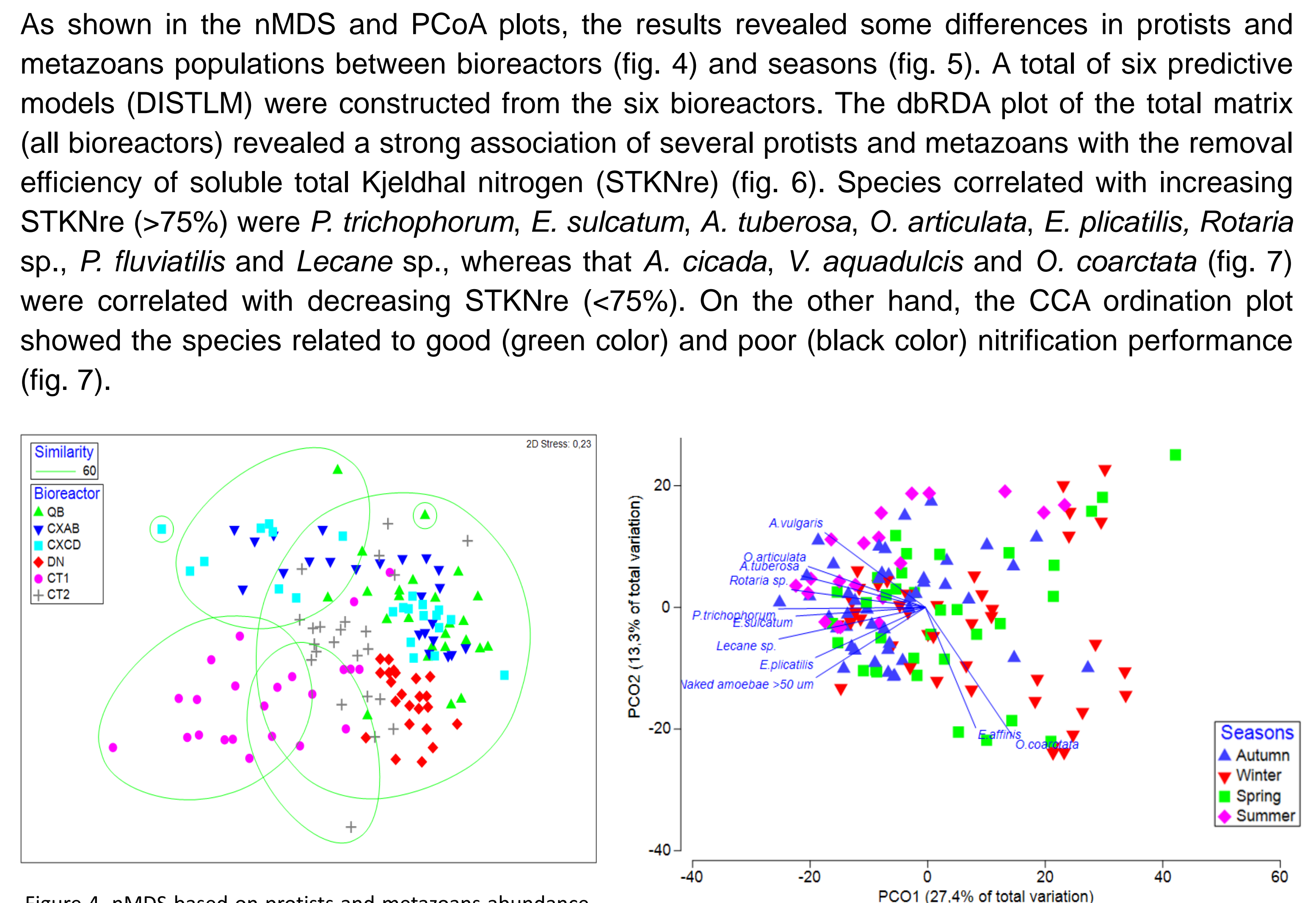

Figure 4. nMDS based on protists and metazoans abundance data, including clusters at 60% of similarity (circles), according to the bioreactor factor.

Figure 5. PCoA based on protists and metazoans abundance data, according to the seasonal factor. Vectors represent Pearson coefficients
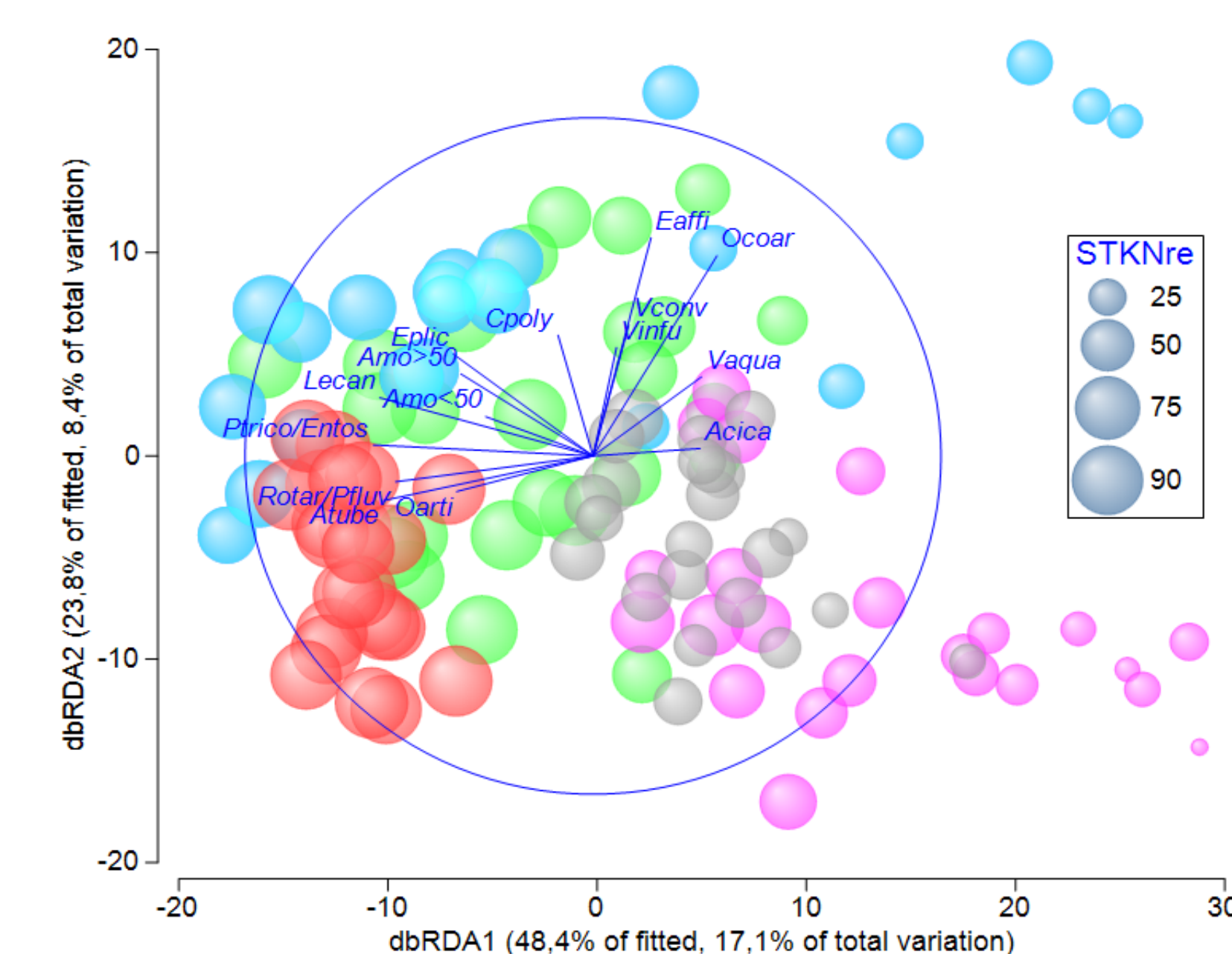

Figure 6. Distance-based redundancy analysis (dbRDA) bubble plot illustrating the DISTLM based on the relationship between STKNre (removal efficiency of soluble total Kjeldhal nitrogen) and the protists and metazoans community structure (bioreactor QB, CXAB, CXCD, DN, CT1 and CT2).
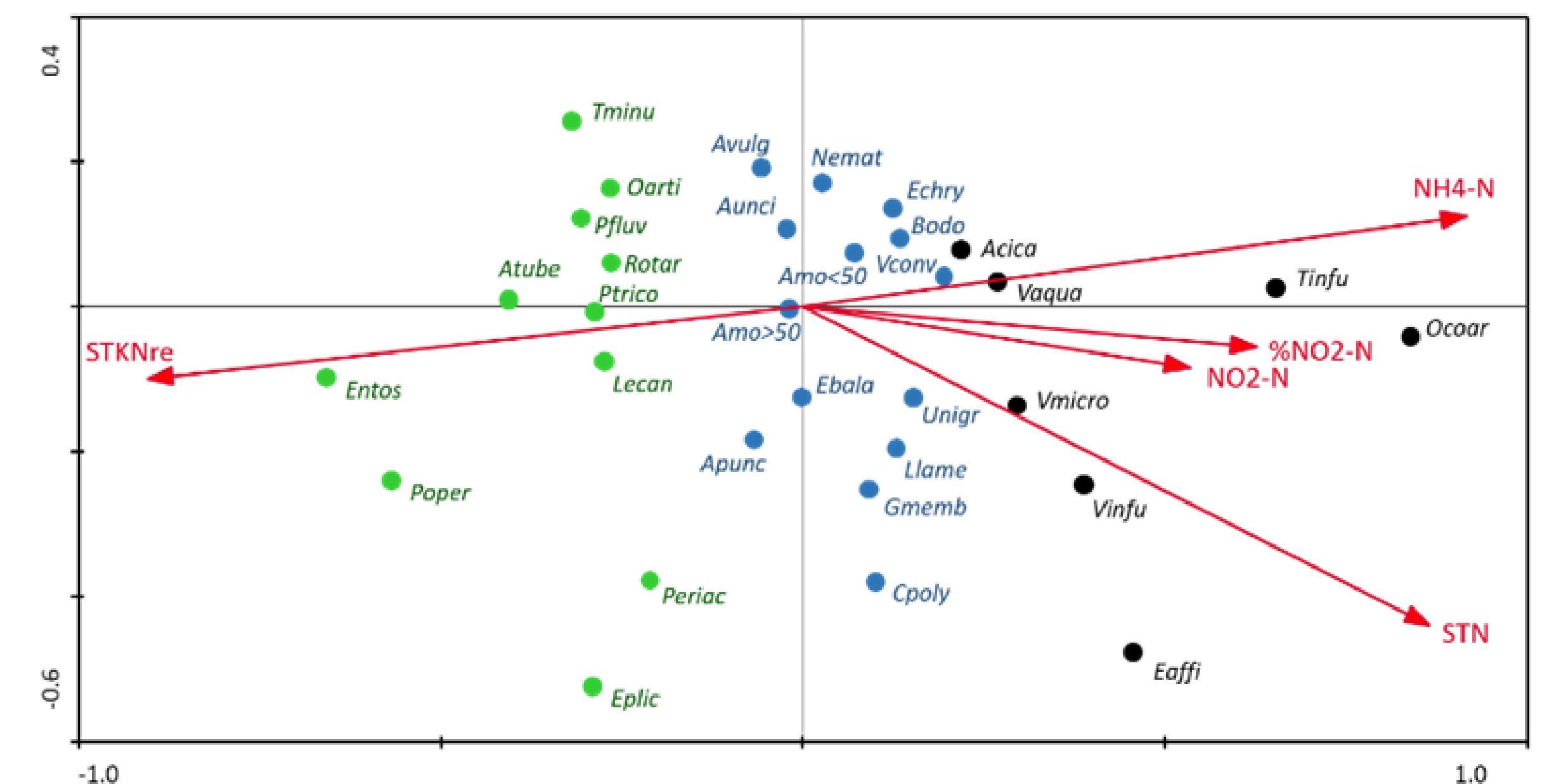

Figure 7. Canonical correspondence analysis (CCA) displaying relation of species to nitrogen compounds. The first two axes explained 69% of the total variation in the data set. Species are indicated by abbreviations (shown in figure 3). STN, soluble total nitrogen (effluent); NO2-N, nitrite nitrogen (effluent); %NO2-N, nitrite nitrogen percentaje (effluent); NH4-N, ammonia nitrogen (effluent). STKNre, removal efficiency of soluble total Kjeldhal nitrogen.
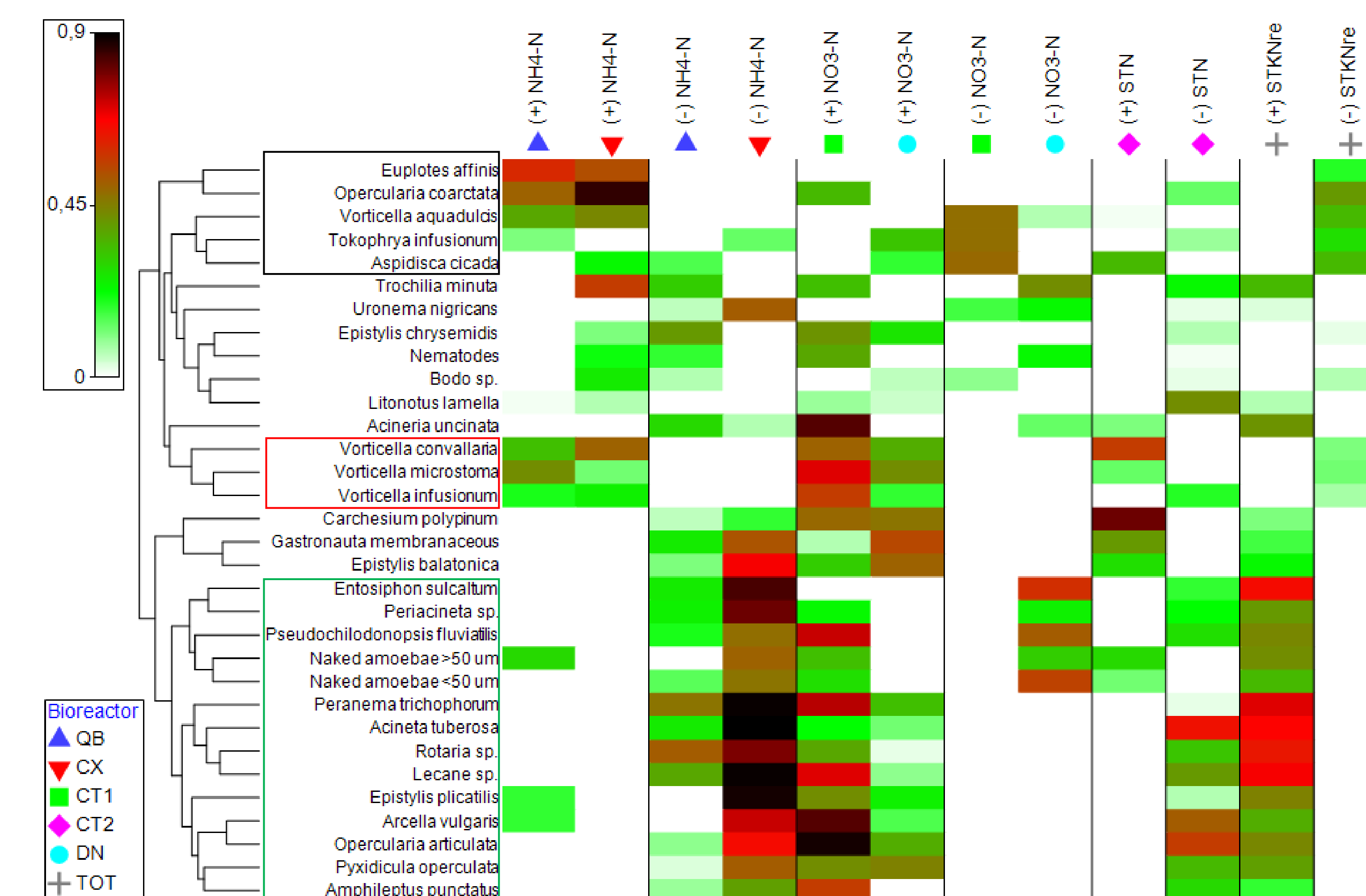

Figure 8. Cluster analysis of the protists and metazoans according to the six best DISTLM. The shade plot illustrates the Pearson correlation coefficients (0-1) of the biological variables with the nitrogen compounds in the effluent. The sign (+) indicates an increase of the compound and the sign (-) indicates a decrease. NO3-N, nitrate nitrogen; NO2-N, nitrite nitrogen; NH4-N, ammonia nitrogen; STN, soluble total nitrogen; STKNre, removal efficiency of soluble total Kjeldhal nitrogen.

The figure 8 shows the cluster analysis of the protists and metazoans according to the six best DISTLM. The shade plot illustrates the Pearson correlation coefficients of the biological variables with the nitrogen compounds most correlated with the dbRDA1 axes of each of the six models. The results revealed three groups of species associated with different nitrogen compounds. Group I was associated mainly with high rates of ammonia nitrogen in effluent and low STKNre (black colour), whereas that group II was associated mainly with high rates of ammonia nitrogen and nitrate nitrogen in effluent (red colour). In contrast, group III was associated with low rates of ammonia nitrogen and soluble total nitrogen (STN) in effluent, and high STKNre (green colour).

Conclusions

The multivariate models constructed with DISTLM and CCA have provided relevant information about the relationships between protists and metazoans and some plant nitrogen compounds, allowing their ecological interpretation and obtaining new bioindicators for monitoring the biological nitrogen removal process in active sludge.

References

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