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Project INHABIT - LIFE08 ENV/IT/000413
Local hydro-morphology, habitat and RBMPs: new measures to improve ecological quality in South European rivers and lakes

WFD and eutrophication assessment: the role of nitrogen as a driving nutrient in shaping phytoplankton assemblages in 13 Italian water bodies.

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The LIFE- INHABIT Project

This study was carried out in the frame of the Project LIFE – INHABIT, started in April 2010 and completed in June 2013. The project aimed at integrating information on local hydro-morphological features into practical measures to improve the reliability of implementation of WFD River Basin Management Plans (RBMPs) in South Europe. The focus was on rivers and lakes that were scrutinized in two areas in Italy, covering a wide range of environmental features and water body types.

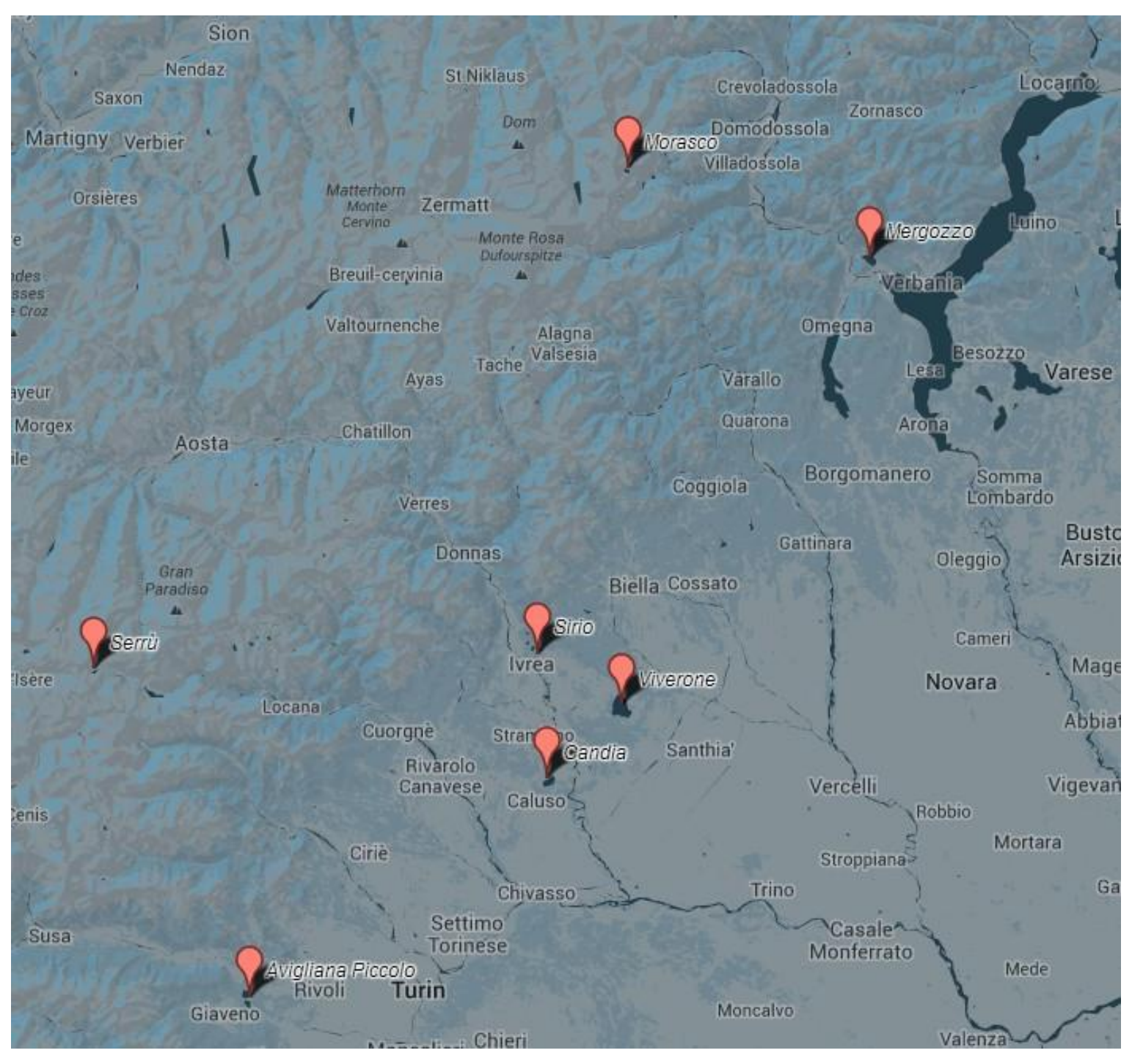
The problem targeted

The enrichment in nitrogen was seen as a possible cause of acidification on aquatic environments, but in recent years the focus has shifted to the role of nitrogen as a limiting factor for algal growth, and thus , as a possible cause of eutrophication, questioning the importance of phosphorus as the only limiting factor of phytoplankton production (Sterner, 2008; Wurtsbaugh & Lewis, 2008). Meta-analysis of experimental data (Elser et al., 1990, 2007) and results of enrichment experiments (Elser et al., 1990) have shown that the limitation by P and N are conditions that can occur with the same frequency. In this context, a factor to take into account are nitrogen inputs from atmospheric deposition, which increased in recent decades due to urbanization, industrialization and intensification of agriculture, which led to a growth of the emissions of nitrogen in the atmosphere (Galloway et al., 2008). Nevertheless, the problem of the nitrogen load to surface waters has been, until now, underestimated. International working groups, born under the ESF Research Networking Programme-'Nitrogen in Europe, have tried to define the amount of nitrogen acceptable for aquatic ecosystems, i.e., the level beyond which it is to be expected a significant damage to the state of the water. This level for nitrates has been identified in 2 mg N l^{-1}, a value often far exceeded in the waters of lakes and rivers in areas with high impact of nitrogen. It was also highlighted that it is not only the form of nitrate-N to monitor and possibly control, but all forms of N, both organic and inorganic, should be monitored as part of the plans of water protection (Sutton et al., 2001).

Methods

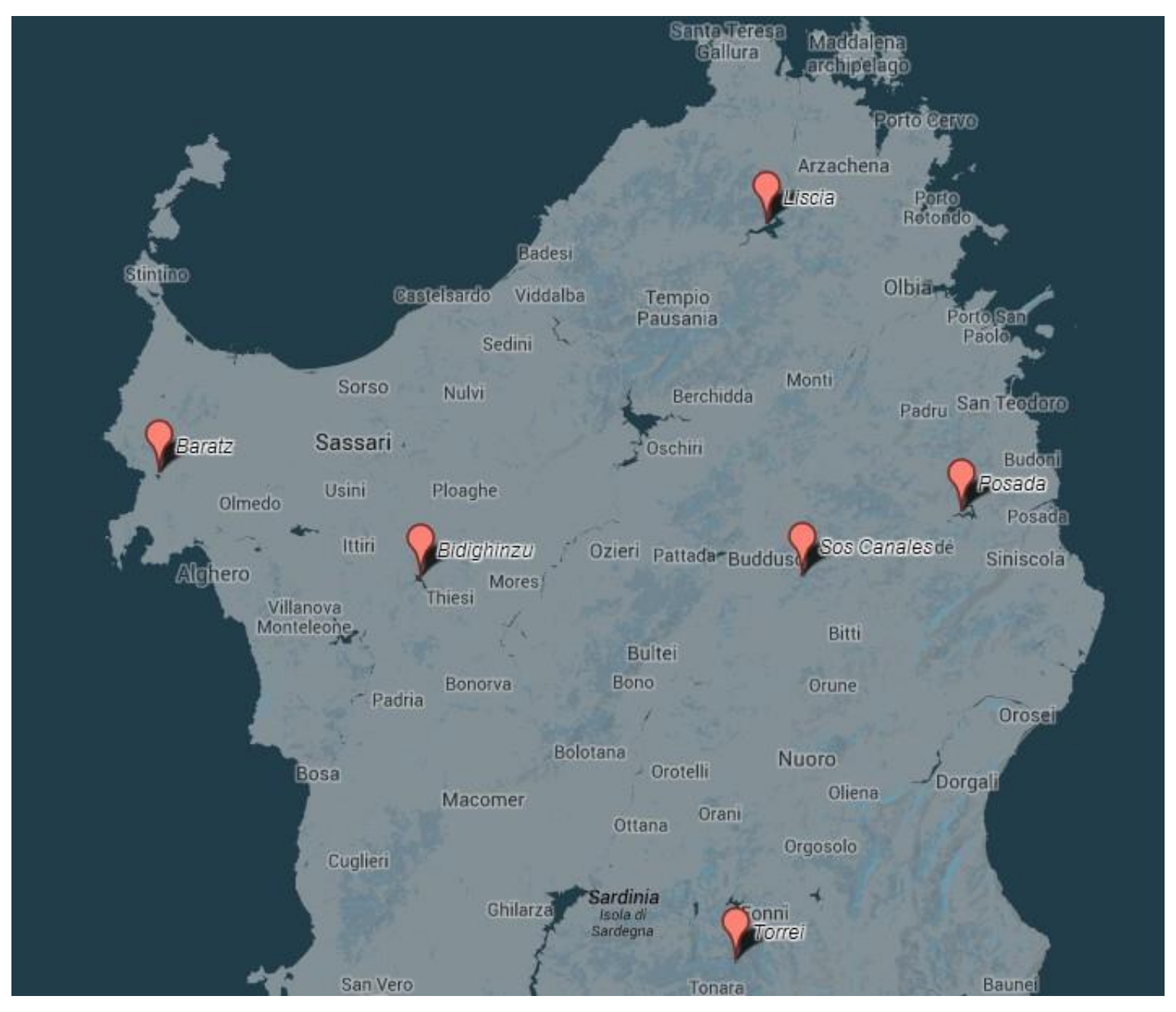
Integrated phytoplankton sampling in the euphotic zone was taken, together with 5 samples for water chemistry, according to stratification (surface, epilimnion, metalimnion, upper and lower hypolimnion), phytoplankton was counted according to inverted microscope technique. The relationships among *taxa* and environmental variables were explored by Canonical Correspondence Analysis and Redundancy Analysis (CCA, RDA; CANOCO 4.5; ter Braak & Smilauer, 2002). The significance of single variables was tested by Monte Carlo test (499 permutations). Generalised Additive Modelling (GAM) was carried out to test the response of single *taxa* to environmental variables, selecting the best fitting model from the AIC value. To simplify the data matrix, the 230 taxa were grouped at the level of 23 orders. The first phase of the analysis has allowed the identification of the orders better correlated with nitrogen, allowing then to select only the algal species belonging to these orders. Further selection was made, eliminating those species not exceeding, as the sum of all the samples, the value of 10 mm^3 m^{-3}. In this way, the number of species in the matrix was reduced to 51.

Study areas



Piedmont Region

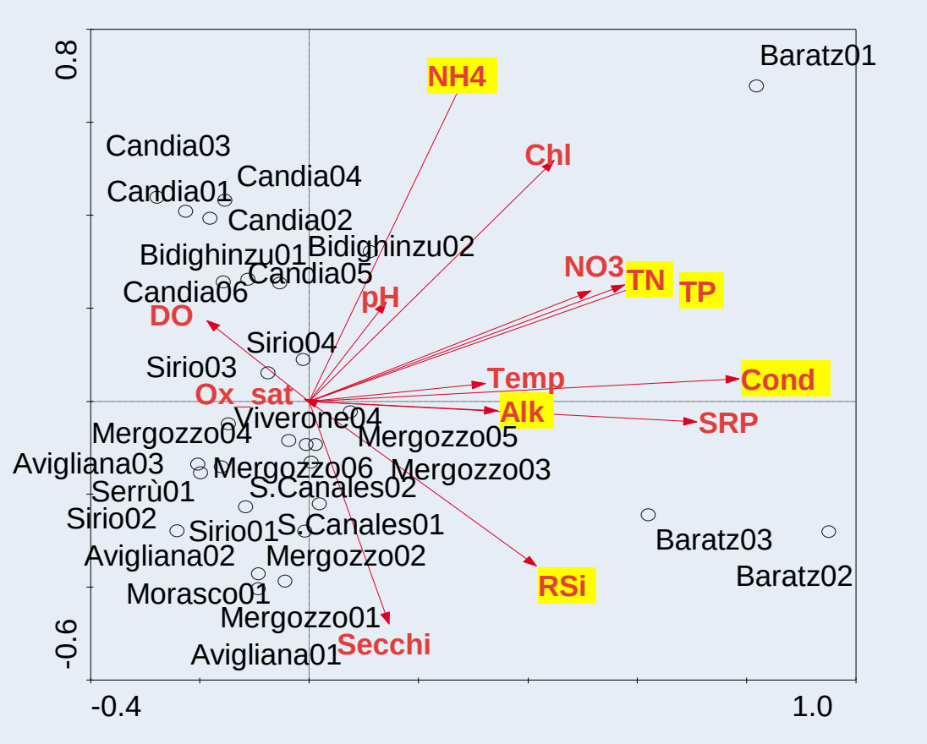
	Lake Morasco	Lake Mergozzo	Lake Candia	Lake Sirio	Lake Viverone	Lake Serrù	Lake Avigliana
Area km^2	0.57	1.83	1.35	0.29	5.58	0.58	0.58
Zmax, Zmean	39, 31	73, 45	8, 5.5	43, 18	50, 22.5	42, 25	12, 7.7
Volume 10^6 m^3	18.2	83	8.1	5.4	125	14.5	4.5



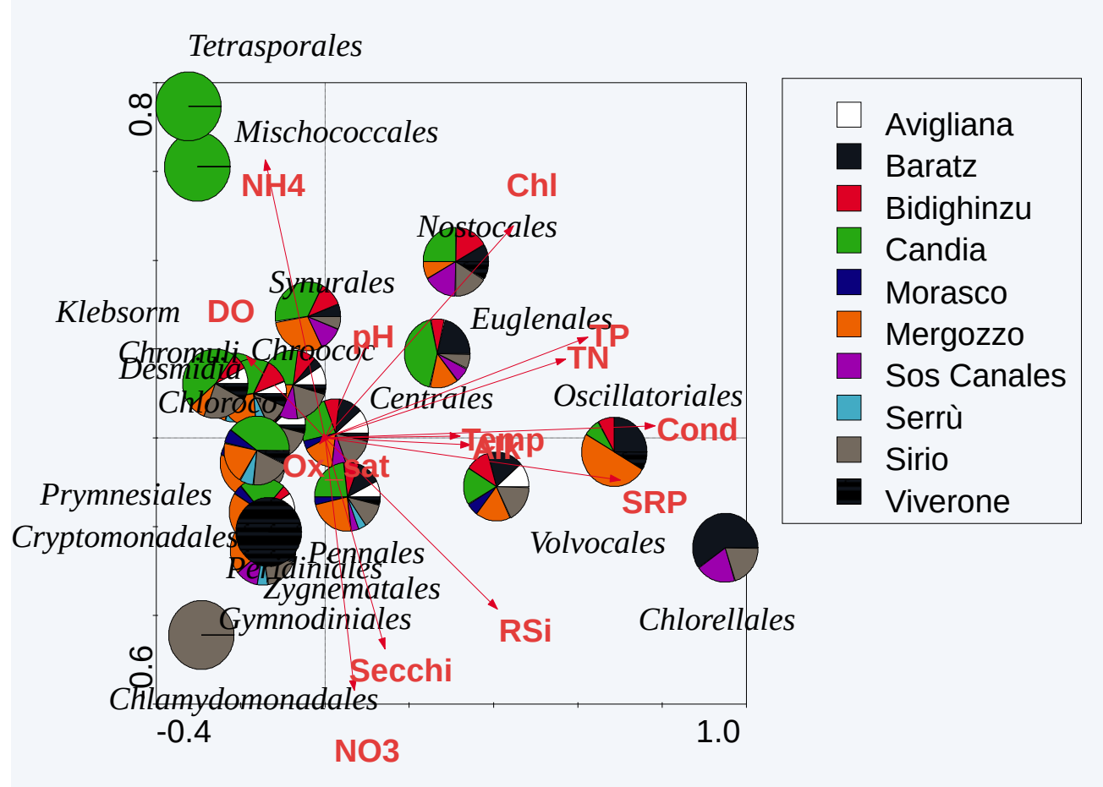
Sardinia Region

	Lake Bidighinzu	Lake Torrei	Lake Baratz	Lake Posada	Lake Sos Canales	Lake Liscia
Area km^2	1.5	0.11	0.6	19	0.3	5.6
Zmax, Zmean	34, -	38, -	11, -	29, -	47, -	63, -
Volume 10^6 m^3	12.6	0.96	2.5	28	4.34	105

Result 1 – Response of phytoplankton orders to N concentration



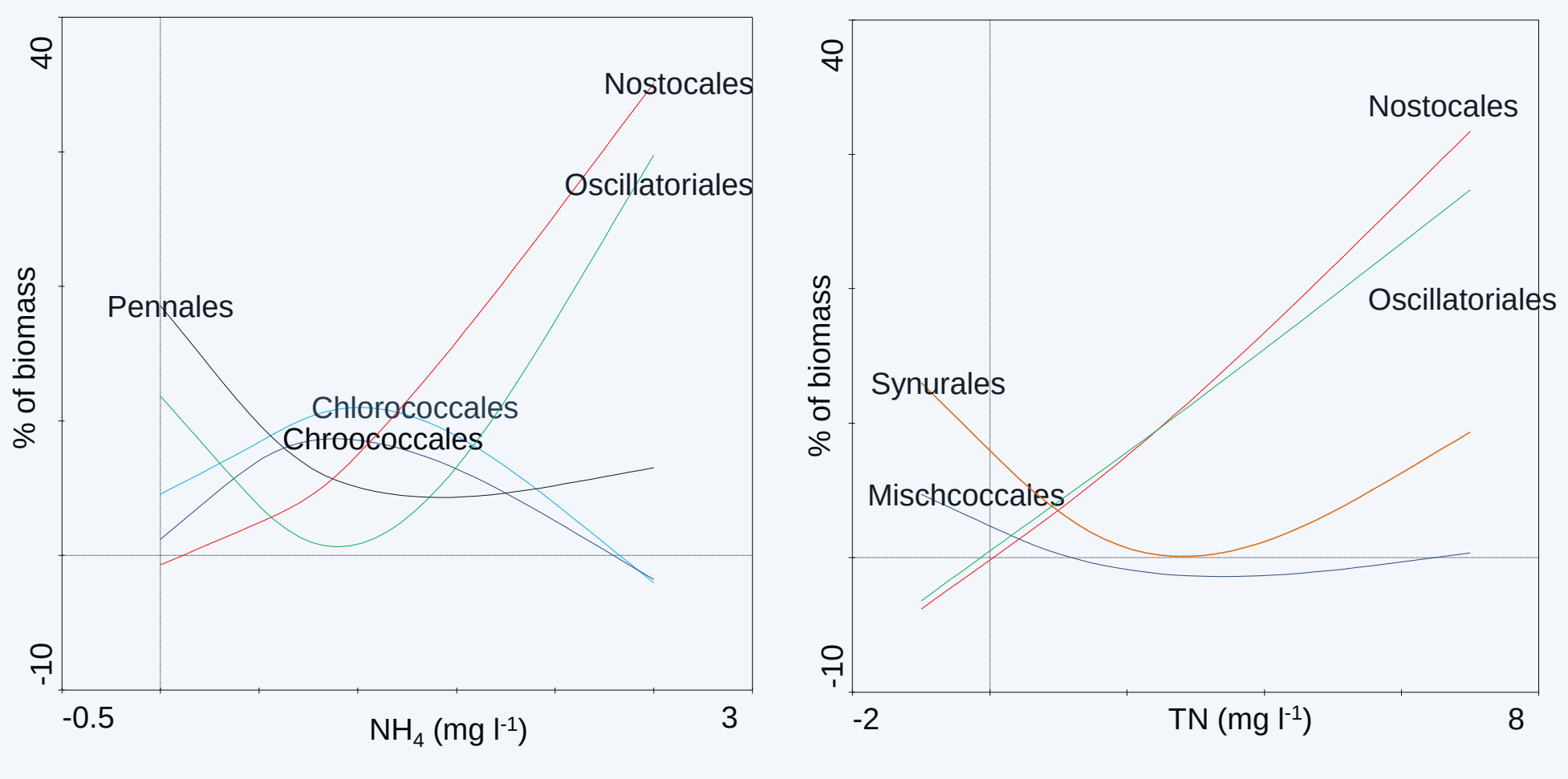
CCA ordination of samples: variables significant after the Monte Carlo test are highlighted.



CCA ordination of phytoplankton orders. Each pie represent the abundance of the order in the lakes (slices).

Phytoplankton orders and significant values of the GA models for NH4 and TN: *p n.l.* indicate the probability of the deviation from linearity in the response. Orders showing a significant response for one or both N-parameters are highlighted.

	NH_4				TN			
Orders	***F***	***p***	***AIC***	***p n.l.***	***F***	***p***	***AIC***	***p n.l.***
Chlorellales					1.53	0.2346	450	----
Chlorococcales	5.42	0.0107	538	0.0028	2.63	0.091	634	0.0748
Chromulinales					2.14	0.1551	868	----
Chroococcales	4.31	0.0242	655	0.0069	2.83	0.1039	737	----
Cryptomonadales	2.84	0.0768	1057	0.0431	3.67	0.0658	1057	----
Desmidiales					2.61	0.1174	456	----
Euglenales	2.09	0.1601	624	----	2.46	0.1052	606	0.0455
Klebsormidiales	2.42	0.1083	144	0.0425				
Mischococcales					8.40	0.0015	40	0.0012
Nostocales	19.02	<0.0001	659	0.0359	26.78	<0.0001	758	----
Oscillatoriales	9.39	0.0008	1264	0.0003	9.79	0.0041	1488	----
Pennales	6.67	0.0046	831	0.0131				
Peridiniales	3.08	0.0967	581	----				
Prymnesiales					2.80	0.0792	568	0.0765
Synurales	2.68	0.1134	545	----	3.77	0.0364	498	0.0108
Tetrasporales	2.73	0.0837	73	0.0514				
Volvocales					1.42	0.2555	1084	----

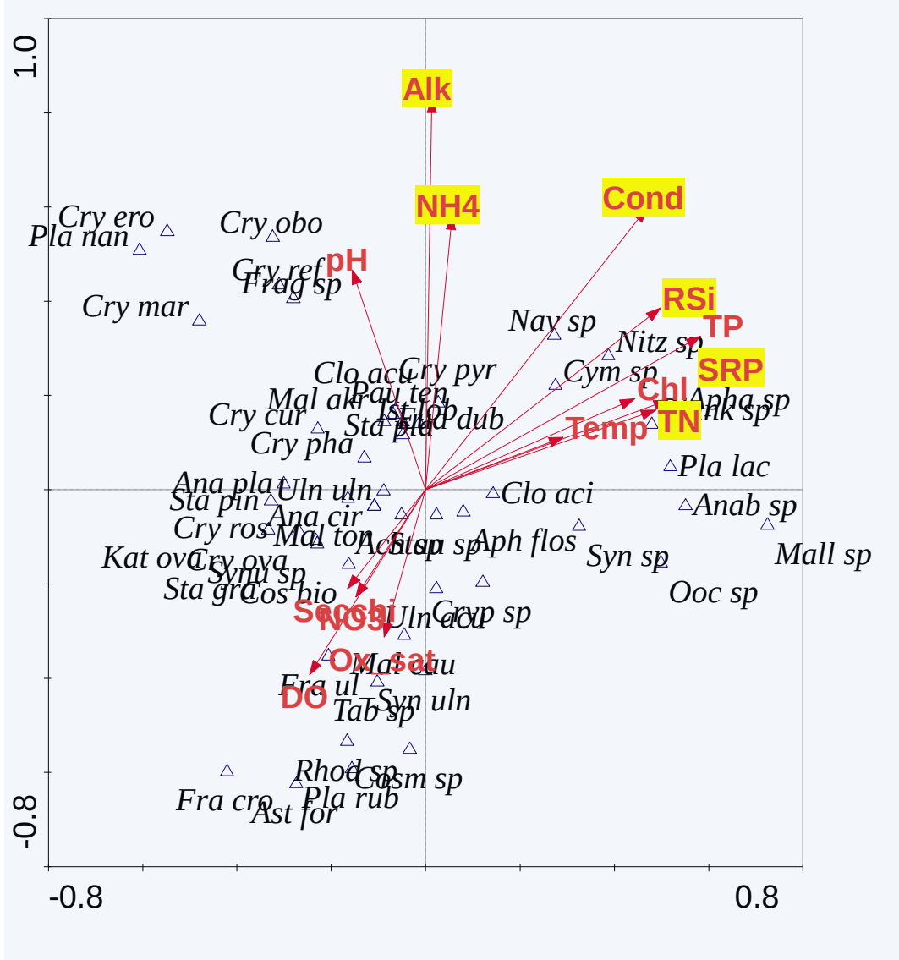


GAM: response of significant phytoplankton orders to NH4 and TN gradients.

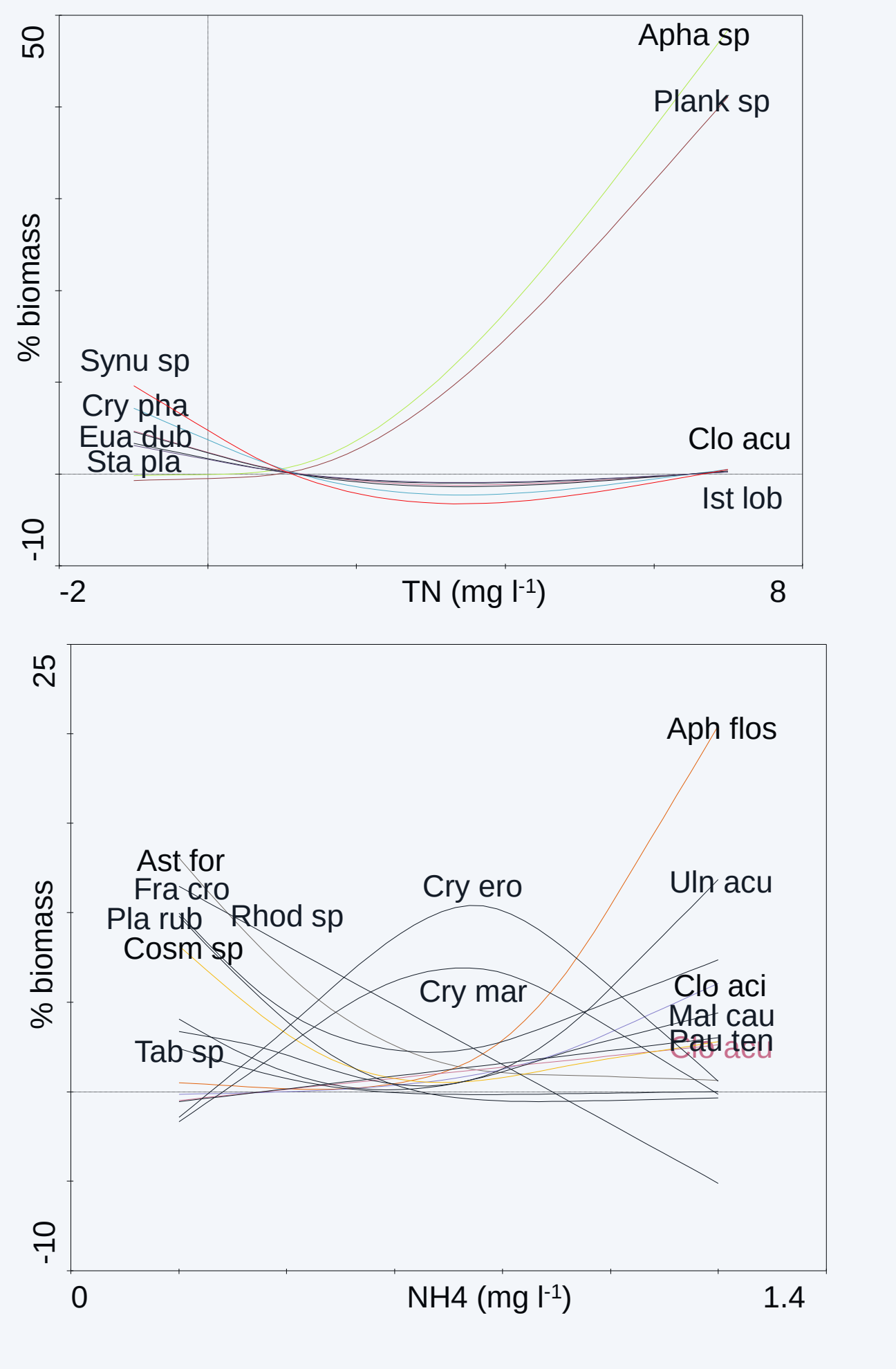
Result 2 – Response of phytoplankton genera/species to N concentration

Phytoplankton genera/species and significant values of the GA models for NH4 and TN: *p n.l.* indicate the probability of the deviation from linearity in the response. Taxa showing a significant response for one or both N-parameters are highlighted. Only those *taxa* belonging to orders significantly correlated with N were testd.

	NH_4				TN			
Genera/Species	***F***	***p***	***AIC***	***p n.l.***	***F***	***p***	***AIC***	***p n.l.***
Aphanizomenon flos aquae	7.16	0.0033	946	0.0056				
Asterionella formosa	4.86	0.0160	1515	0.1268				
Closterium aciculare	7.31	0.0030	84	0.0211				
Closterium acutum	7.23	0.0122	64	----	5.01	0.0145	63	0.010
Cosmarium sp.	6.7	0.0045	473	0.0096				
Cryptomonas erosa	6.59	0.0048	1515	0.0084				
Cryptomonas marssoni	7.5	0.0027	660	0.0044				
Cryptomonas reflexa	2.34	0.1168	535	0.1028				
Fragilaria crotonensis	10.98	0.0026	1215	----				
F.ulna var. angustissima	2.55	0.1216	478	----				
Fragilaria sp.	2.05	0.1633	493	----				
Isthmochloron lobulatum	2.71	0.0854	41	0.0328	4.57	0.0200	37	0.0129
Mallomonas akrokomos	3.69	0.0652	424	----				
Komma caudata	4.01	0.0304	296	0.0093				
Paulschulzia tenera	5.83	0.0228	107	----				
Plagioselmis nannoplanctica	2.81	0.0784	1054	0.0681				
Planktothrix rubescens	8.91	0.0011	631	0.0231				
Rhodomonas sp.	4.81	0.0166	741	0.0100				
Synedra ulna	2.58	0.1199	286	----				
Tabellaria sp.	3.58	0.0424	101	0.0779				
Ulnaria acus	3.93	0.0321	691	0.0095				
Ulnaria ulna	3.33	0.0793	353	----				
Aphanizomenon sp.					156	<0.001	164	<0.001
Cryptomonas ovata					2.53	0.0994	684	0.0979
Cryptomonas phaseolus					8.38	0.0016	94	0.0014
Cryptomonas pyrenoidifera					2.84	0.0767	140	0.0452
Cryptomonas rostrata					3.23	0.0559	222	0.0297
Euastrum dubium					7.8	0.0022	41	0.0017
Katablepharis ovalis					2.67	0.0881	772	0.0762
Nitzschia sp.					2.34	0.1375	834	----
Planktothrix sp.					5200	<0.001	1327	<0.001
Staurastrum pingue					2.22	0.1288	335	0.0917
Staurastrum planctonicum					3.68	0.0392	39	0.0248
Synura sp.					6.15	0.0065	241	0.0046

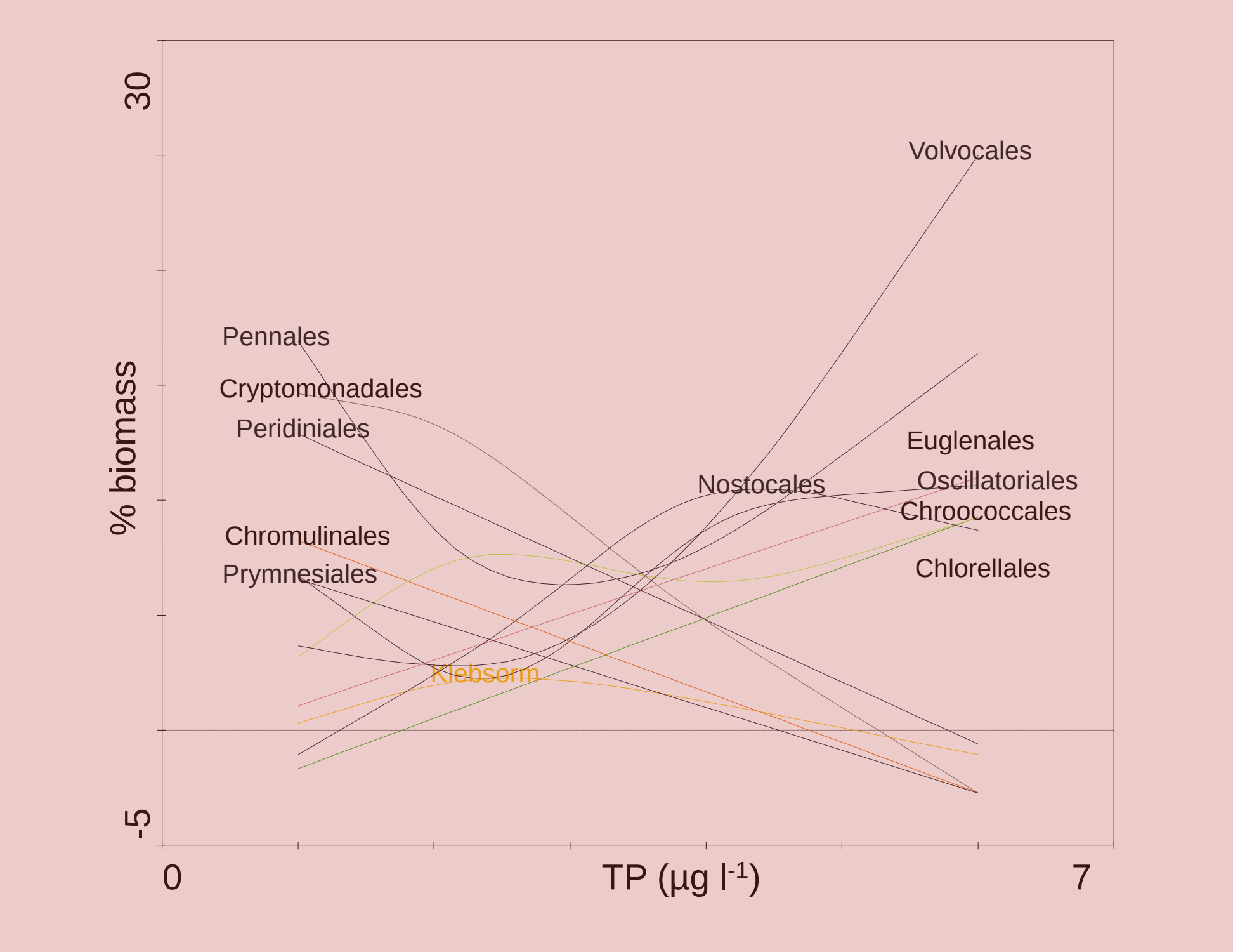


RDA ordination of genera/species: variables significant after the Monte Carlo test are highlighted.



GAM: Response of significant phytoplankton *taxa* to TN and NH_4 gradients. *Taxa* codes report the first three letters of generic and specific names (see table).

Result 3 – Response of phytoplankton orders to TP concentration



The response to TP was analysed to evaluate if the relationship with N-compounds was particular for cyanobacteria, or they responded in the same way to a second nutrient. The results show that many orders are affected by TP concentration, not only cyanobacteria. This confirms the key role of nitrogen in controlling this group

Conclusions

Considering phytoplankton in general, ammonium seems to be the preferred source of nitrogen by nitrogen-fixing cyanobacteria (Blomqvist et al., 1994): our results confirm cyanobacteria are dominant at the highest levels of ammonia nitrogen.

In the group of lakes studied, an increase of cyanobacteria belonging to Nostocales and Oscillatoriales, following the increasing total nitrogen availability (not only as ammonium), was observed. Since the role of nitrogen-fixing cyanobacteria in promoting an increase in the concentrations of nitrogen, was negligible in many lakes (Lewis & Wurtsbaugh, 2008), it can be inferred that its increase in surface waters, due to human activities, could be responsible for an increased importance of cyanobacteria, even in environments where phosphorus concentrations are moderate (Jeppesen et al., 2011).

The relationship observed between the concentration of ammonium and pennate diatoms, both at the order and species level, seems to confirm what described by Domingues et al. (2011), who observed an inhibitory effect of ammonia on the growth of this algal group, suggesting a possible toxic effect.

The results of our analysis, further emphasize the need to pay more attention to the contributions of nitrogen, growing steadily in recent decades: the effects of the increased nitrogen load on aquatic ecosystems have been , until now, poorly studied, because the attention was focused primarily on phosphorus (Elser et al., 2009a). However, in view of the adoption of actions aimed at reducing the input of phosphorus, nitrogen can become a key controlling factor, affecting phytoplankton growth and assemblage structure.

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