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The influence of land use and the nature of the substrate on the diatom association from ponds found in two adjacent regions of France

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Resumé – **Influence de l'occupation des sols et de la nature du substrat sur les associations de diatomées collectées dans les mares de deux régions adjacentes de France.** Les diatomées provenant de 140 mares de deux régions de France, le plateau calcaire de la Beauce au nord et la région de sable et d'argile d'Orléans et de la Sologne au sud, ont été analysées afin d'évaluer l'influence de la géologie régionale, de l'aménagement du territoire et des substrats colonisés. Des mares permanentes et éphémères ont été inclues dans les analyses. La procédure de permutation multi-réponses illustre les différences et similarités entre les deux régions, les 13 différentes classes de types de mares et les différents substrats. Les analyses ont démontré pourquoi certains types devraient être regroupés dans une large classe, avec six types de mares retenus. La plupart des catégories de substrats n'ont montré aucune différence significative ; seulement une catégorie, les sphaignes, diffère de toutes les autres, tandis que la boue et les racines vivantes diffèrent également beaucoup des autres catégories.
Les analyses des correspondances canoniques des deux régions ensemble et puis de chacune des régions séparément, en utilisant uniquement les données des mares permanentes, ont permis de mettre en évidence les principaux facteurs environnementaux. Pour les deux régions, trois facteurs essentiels ont été déterminés : le pH, le log conductivité et l'aire de surface. Le plateau calcaire de la Beauce est influencé par le pH et le potassium, tandis que la région de sable et d'argile d'Orléans et Sologne est influencée par le log conductivité, et SiO_2 sur les axes 1-2.

Diatomées / mares / occupation des sols / comparaison de substrat / comparaison entre régions (Beauce, Orléans-Sologne)

Abstract – Diatoms coming from 140 ponds ("mares") from two regions of France the Calcareous plateau of the Beauce in the north and the sand and clay region of Orléans and Sologne in the south were analyzed for the influence of regional geology, land use and growth support. Both permanent ponds and ephemeral ponds were included in the analyses. Multi-response permutation procedure illustrated the differences and similarities between the two regions, the 13 different classes of pond type and different substrates. The analyses demonstrated why certain types should be regrouped into one large class, with six pond types retained. Most of the substrates categories showed no significant differences but one category,

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Sphagnum (peat moss) was different from all the others while mud and living roots also differed from many of the other categories.
Canonical correspondence analyses of both regions together and then each region separately using only the permanent pond data resulted in different environmental factors explaining species ordination. For the two regions combined three essential factors were determined; pH, log conductivity, and pond surface area. The Beauce calcareous plateau was influenced by pH and K while the sand and clay region of Orléans and Sologne were influenced by log conductivity, and SiO_2 on axis 1-2.

Diatoms / ponds / land use / substrate comparison / regional comparison (Beauce, Orléans-Sologne)

INTRODUCTION

Ponds have many different uses: water for cattle, irrigation, fish ponds, runoff retention or used to increase infiltration. Farmland ponds support a variety of aquatic organisms and often contain rare or unique species and could add to regional biodiversity (Declerck *et al.*, 2006). Their smaller volume to edge ratio can create a greater littoral zone, have a larger impact from tree shading or increased direct exchange of matter and organisms with the terrestrial matrix (Declarck *et al.*, 2006). The shallow depth may allow more light conditions for vegetation, thereby a more intensive benthic-pelagic coupling (Declerck *et al.*, 2006). The ponds are rich in biodiversity and often can be biodiversity "hot spots" (Céréghino *et al.*, 2008, Davies *et al.*, 2008, Williams *et al.*, 2003). Biodiversity has been examined for aquatic plants (Williams *et al.*, 2003, Oertli *et al.*, 2002), and for Gastropoda, Sphaeriidae, Coleopteran, Odonata and Amphibia and it was found that smaller ponds had more species (Oertli *et al.*, 2002).

Ponds as used in this article are defined as having a surface area between 50 and 5000 m^2 with a maximum depth of two meters and the lack of artificial drainage (Sajaloli & Dutilleul, 2001). They differ from marshlands which can be drained. Ponds are located throughout all of France (about 60,000 ponds, Bertrand *et al.*, 2013) varying from forest area, agricultural plains, to urban and sub-urban setting and some situated along country roads and freeways. All have been formed by man for his benefits thereby having an anthropization effect with different materials and chemicals entering in the ponds according to their location. Precipitation, solar insolation and temperature vary throughout the year, causing depth changes with either over flow into other areas or in certain case total disappearance of the pond. Water temperature can vary from 36 °C to a partially or totally frozen pond (Bertrand *et al.*, 2013). Variations occur in vegetation cover, light penetration, types of substrates, varying amounts of decomposing material, dissolved oxygen and other chemicals, each of these factors often changing daily or with seasons.

Diatoms can be found living in a variety of different physical and chemical enivronments, along with different hydrological conditions and habitats (Lowe, 1974; Van Dam *et al.*, 1994; Hay *et al.*, 2000; Pan *et al.*, 2006; Reid & Ogden, 2009). Until recently, investigation pond diatoms have been very limited (Bertrand *et al.* 2013, 2014a, b, Poulíčková *et al.* 2009, Špačková *et al.* 2009). Pond water quality could become important in the future as water shortages are bound to develop as seen in other parts of the world. This study expands on recent studies (Bertrand *et al.* (2013, 2014, 2015) using the same data base. As was discussed in Bertrand *et al.* (2013), there is no accepted procedure for sampling ponds comparable with the protocol developed for the analysis of river water quality using diatoms. Therefore a better understanding of diatoms found in the ponds is needed in order to develop a water quality index. Nine different types of substrates were analyzed to determine which substrates would be most representative of the diatom communities found in ponds. This could later be used in developing a synthetic index for evaluating pond water quality. Also examined were the differences and similarities between the two regions, the effect of vegetation types surrounding the pond, and diatoms related to permanent and ephemeral ponds.

MATERIAL AND METHODS

Two regions of France were studied starting in the northeast at Aschères-le-Marché on the calcareous plateau region of the Beauce and finishing in the sand and clay region of Ligny-le-Ribault, Sologne in the southeast. The ponds are located on various substrates but the substrate types were only established after prospecting and, therefore, site selection was based on their location within the regions and from prospecting in the field. Ponds were classified according to surrounding land use based on Sajaloli & Dutilleul (2001). A total of 13 categories based on anthropogenic pressure were recognized in the present study area: (1) surrounded by a deciduous forest, or (2) a pine forest, (3) within a coppice, (4) at the edges of a forest, (5) within a logging forestry zone, (6) associated with heath lands and moors, (7) beside a freeway, or (8) a country road, (9) within a prairie, or (10) a fields, (11) exterior to a group of buildings, (12) at the edge of villages, or (13) inside a village.

Many of the ponds lacked natural or artificial lithic substrates that are used in standardized river sampling. For this reason, any available and accessible submerged substrate was used along with plankton and several samples if possible from the same pond were collected. Table 1 lists the different sampled categories and the method of collection.

Table 1. The 12 categories sampled and method of collection used in this study

Substrate types	*Method of collection*
Mud	Scraping of about 2 cm^2 of the surface pellicles or area where diatoms were suspected to be present.
Rocks	Picking 4 or 5 small rocks preserved with pond water.
Wall	Scraping of a small surface area approximately cm^2.
Living submerged plant roots	Collecting a portion of about 12 cm.
Aquatic plants	Taking two sections, one part of the living portion of the plant and another from a dead portion.
Large living submerged stems	Sampling from a living section.
Grasses	Sampling from the submerged section.
Dead branches and stems	Removing 8 to 10 cm from the submerged section or taking 5 cm of small branches in avoiding colony destruction.
Dead submerged leaves	Sampling only leaves that were on the bottom of the pond.
Submerged mosses	Sampling both living and dead sections.
Submerged Sphagnum	Collecting a living portion and a dead part of the distal extremities.
Plankton	Collected surface water.

After collection, samples were stored in 150 cm^3 glass bottles with no preservative added. Surface temperature and conductivity were measured (in the field) using a Hanna – HI 99300 and pH was determined (in the lab) by colorimetry within 24 hours after the water sample collection using the test Visocolor-Eco of Macherey-Nagel**.** Surface area was estimated by measuring dimensions (maximum length and width) and by applying a coefficient of shape (oval, trapezoid, rectangular, indeterminate, etc). Thirteen chemical variables {Cl (mgL^{-1}), SO_4 (mgL^{-1}), K (mgL^{-1}), Fe (mgL^{-1}), NO_2 (mgL^{-1}), NH_4 (mgL^{-1}), Mg (mgL^{-1}), Ca (mgL^{-1}), $CaCO_3$ (°f, alkalinity in French degrees), PO_4 (mgL^{-1}), NO_3 (mgL^{-1}), and SiO_2 (mgL^{-1})} were analyzed within 24 hours in the lab. The following chemical data was missing for the ephemeral ponds: Cl, SO_4, K, Fe, NO_2, NH_4, Ca, Mg, and conductivity. Therefore, the ephemeral ponds were excluded from the analyses of major environmental factors affecting each region.

All equal parts of raw material were first placed in 30% H_2O_2 for 24 hours to dissolve the mucus surrounding diatom cells encased in the substrate. The samples were then agitated for 5 minutes before extracting the supernatant for later use. The substrate was washed two more times and then the extracted liquid is added to the supernatant. The combined liquid is then filtered and H_2O_2 added to the liquid in an equal amount as the liquid. This was boiled until a whitish brown residue was obtained. The brown residue was then treated with HCl to remove any carbonates. Finally the samples were rinsed three times in demineralized water. This method allowed for the complete removal of diatoms from porous substrates, such as wood or rocks and the dissolution of *Sphagnum* (peat moss). After treating the samples, a drop of the digested material was deposited on a cover slip for drying. In order to eliminate the last traces of organic matter, particularly for *Sphagnum,* the cover slip was placed on a hotplate which was turned to maximum heat setting. The cover slip was left on the hotplate for 120 minutes, then the hot plate was turned off and the cover slip was mounted using Naphrax.

Diatoms (examined under 100 × magnification) were identified using Germain (1981), Krammer & Lange-Bertalot (1986, 1988, 1991a, b), Krammer (1992) and Lange-Bertalot (1993). Between 386 and 533 diatoms were counted with the exception of two samples which only had 269 and 288 diatom identified. Multi-response permutation procedure (MRPP), indicator species (IS) and canonical correspondence analyses (CCA) were performed using Pc-Ord (McCune & Mefford, 2006). MRPP and IS used the relative percentage of all species identified in a sample. The following options were selected for the MRPPs: distance measure – Sorensen (Bray-Curtis), rank transform distance matrix, pairwise comparisons, and weighting of groups – n/sum(n) (recommended). For the IS, the following options were selected: quantitative or binary response Dufrêne & Legendre ISA Eqn 1, randomization – source of random number seeds (time of day) and number of runs 999. The CCA was run using only the species occurring in at least one sample with a relative abundance of ≥ 1%. The CCA options chosen were column scores standardized by centering and normalizing, scaling of ordination – comprise representation of species and samples, scores for graphing sample scores are linear combination of factures, randomization test on, test null hypothesis – no relationship between matrices, and number of runs 999. The computer program R (http://www.r-project.org/)was used to determine the multi-collinearity using the vegan package.

RESULTS

From the 290 sample, 382 taxa were identified to the species level. No indetermined species were found. According to Denys (1991/2), eight lifeforms were categorized but these categories were not explored as the object of the study was to find what substrates were best to sample for evaluating pond water quality. Another reason for not looking as lifeform category such as planktonic and benthic because these can be broken down in to even more catorgies, take for example plantonic can be euplanktonic, ortychoplanktonic of epontic orign or tychoplanktonic of benthic orgin.

MRPP and IS analysis

In MRPP, the greater negative is T, the larger the separation between two groups. For the MRPP of permanent ponds versus ephemeral ponds, there was very little difference between the two (T = –3.89, p = 0.003). However, certain species were found in 100% of the sites, either in permanent or ephemeral ponds (Table 2), but many of these species were found in low relative abundance in these sites while others species occurred commonly within either pond type. However, there was a large disparity between the numbers of species occurring in > 90% of permanent ponds (70 species) compared to ephemeral ponds (9 species). Nevertheless, the IS had no observed indicator value of > 50%, except for *Gomphonema gracile* Ehrenb. with IS value of 58.2%, p = 0.002, for permanent ponds. There were also certain species specific to one region over another. The Orléans – Sologne region had 34 species (T = –18.09, p = 0.0001) occurring in > 90% while the Beauce region had only 27 species (Table 3).

A MRPP comparison between the different pond classes including dry samples had a T = –28.99 and p = 0.0001 (Table 4A – 13 pond classes). T values greater than –10.00 occurred between related forest areas (deciduous forests, pine forests, and forestry zones), coppices, and heath lands/moors versus prairies, fields, and areas associated with villages. Coppices and pine forest versus freeways/toll ways also had T > –10.00. Edge of forest was not significantly different from deciduous and pine forests, heath land/moors, and country roads, while freeways/toll ways had significant different from areas related to villages (exterior to building, edge of villages and interior villages). No significant difference was observed between the anthropologically related pond classes (exterior to building, edge of villages and interior of villages.

A new analysis was undertaken after grouping several classes into six classes (forests, coppices, heath lands/moors, roads, prairie/fields and villages). Forests included deciduous forests, pine forests, edges of forests and forestry areas. Roads regrouped both country roads and freeways/toll ways, and villages grouped together exterior buildings, edge of villages, interior of villages. A more negative T value (T = –40.00, p = 0.001) was obtained using this classification. The new pond classes showed greater separation between the different groups (Table 4B – 6 pond classes). Forest, coppices, and heath lands/moors had the greatest separation from prairies/fields and villages. Roads had the least separation from all the other classes. When roads were plotted using CCA (Fig. 1), it showed a wide distribution through all the other classes and, therefore, most likely should be reclassified according to land use surrounding the pond. Interestingly, some species were only found in a certain pond class and were observed in > 90% of the sites belonging

Table 2. Species found in greater than 90% in occurrence in either permanent or ephemeral ponds from both regions

Permanent ponds	%	*Permanent ponds – cont.*	%
Achnanthes biasolettiana Grunow	100	*Navicula cincta* (Ehrenb.) Grunow	100
Amphipleura pellucida (Kütz.) Kütz.	100	*Navicula festiva* Krasske	100
Aulacoseira crenulata (Ehrenb.) Thwaites	100	*Navicula mediocris* Krasske	100
Aulacoseira granulata (Ehrenb.) Simonsen	100	*Navicula perminuta* Grunow	100
Brachysira brebissonii R.Ross	98	*Navicula radiosa* Kütz.	94
Brachysira neoexilis Lange-Bert.	100	*Navicula radiosafallax* Lange-Bert.	100
Cocconeis placentula Ehrenb.	98	*Navicula seminulum* Grunow	100
Cyclostephanos dubius (Fricke) Round	100	*Navicula suchlandtii* Hustedt	100
Cyclotella meneghiniana Kütz.	100	*Navicula wildii* Lange-Bert.	100
Cyclotella pseudostelligera Hustedt	100	*Neidium alpinum* Hustedt	100
Cymbella caespitosa (Kütz.) Brun	100	*Neidium productum* (W. Smith) Cleve	100
Cymbella cesati (Rabenh.) Grunow	100	*Nitzschia communata* Grunow	100
Cymbella falaisensis (Grunow) Krammer & Lange-Bert.	100	*Nitzschia draveillensis* Coste & Ricard	100
Cymbella gracilis (Ehrenb.) Kütz.	100	*Nitzschia fonticola* Grunow	100
Cymbella mesiana Cholnoky	100	*Nitzschia graciliformis* Lange-Bert. & Simonsen	100
Cymbella microcephala Grunow	100	*Nitzschia modesta* Hustedt	100
Cymbella prostrata (Berkeley) Cleve	100	*Nitzschia prolongata* Hustedt	100
Cymbella tumida (Bréb) Van Heurck	100	*Nitzschia pseudofonticola* Hustedt	100
Epithemia adnata (Kütz.) Brébisson	100	*Nitzschia pumilla* Hustedt	100
Epithemia sorex Kütz.	100	*Reimeria sinuata* v. *delognei* (Grunow) Lange-Bert.	100
Epithemia turgida (Ehrenb.) Kütz.	100	*Nitzschia sociabilis* Hustedt	100
Eunotia arcus Ehrenberg	100	*Pinnularia brauniana* (Grunow) Mills	100
Eunotia exigua (Bréb) Rabenhorst	98	*Pinnularia brebissonii* Kütz.	100
Eunotia fallax A. Cleve	100	*Pinnularia interrupta* W. Smith	100
Eunotia incisa Gregory	99	*Pinnularia legumen* Ehrenb.	100
Eunotia paludosa Grunow	92	*Rhopalodia gibba* (Ehrenb.) O. Müller	100
Eunotia romboidea Hustedt	100	*Stenopterobia curvula* (W. Smith) Krammer	100
Fragilaria bidens Heiberg	100	*Surirella brebissonii* Krammer & Lange-Bert.	100
Pseudostaurosira brevistriata (Pant.) Edlund	100	*Tabellaria fenestrata* (Roth) Kütz.	97
Staurosira venter (Ehrenb.) Cleve & Möller	100	*Thalassosira pseudonana* Hasle & Heimdal	100
Ulnaria acus (Kütz.) M.Aboal	97		
Fragilaria crotonensis Kitton	94	*Ephemeral ponds*	%
Frustulia saxonica Rabenh.	100	*Achnanthes rupestroides* Hohn	100
Gomphonema acuminatum Ehrenb.	96	*Craticula ambigua* (Ehrenb.) D.G.Mann	90
Gomphonema augur Ehrenb.	95	*Diadesmis contenta* (Grunow) D.G.Mann	95
Gomphonema angustum Agardh	100	*Eunotia septentrionalis* Oestrup	93
Gomphonema gracile Ehrenb.	99	*Fragilaria crotonensis* Kitton	100
Gomphonema minutum (Agardh) Agardh	100	*Hantzschia amphioxys* (Ehrenb.) Grunow	90
Gomphonema subclavatum Grunow	100	*Luticola goeppertiana* (Bleisch) D.G.Mann	97
Gomphonema truncatum Ehrenb.	100	*Navicula accomoda* Hustedt	95
Hantzschia elongata (Hantzsch) Grunow	100	*Pinnularia brevicostata* Cleve	92

Table 3. List of species found in greater than 90% only in Orlean-Sologne region or the Beauce

34 species – Orleans et Sologne	%	*27 species – Beauce*	%
Aulacoseira crenulata (Ehrenb.) Thwaites	100	*Achnanthidium catenatum* (Bily & Marvan) Lange-Bert.	92
Aulacoseira granulata (Ehrenb.) Simonsen	100	*Achnanthes rupestroides* Hohn	100
Cocconeis placentula Ehrenb.	93	*Amphipleura pellucida* (Kütz.) Kütz.	92
Craticula halophyla (Grun) D.G.Mann	94	*Amphora pediculus* (Kütz.) Grunow	90
Cyclotella pseudostelligera Hustedt	100	*Brachysira neoexilis* Lange-Bertalot	100
Eunotia arcus Ehrenb.	100	*Cyclostephanos dubius* (Fricke) Round	100
Eunotia fallax A. Cleve	96	*Cymbella caespitosa* (Kütz.) Brun	100
Eunotia naegelii Migula	95	*Cymbella prostrata* (Berkeley) Grun	98
Eunotia paludosa Grunow	91	*Cymbella tumida* (Bréb) Van Heurck	100
Eunotia steineckii Petersen	90	*Eunotia septentrionalis* Oestrup	97
Pseudostaurosira brevistriata (Pant.) Edlund	100	*Fragilaria bidens* Heiberg	100
Fragilaria crotonensis Kitton	100	*Navicula capitatoradiata* Germain	94
Fragilaria elliptica Schumann	100	*Navicula perminuta* Grunow	97
Fragilaria nanana Lange-Bert.	100	*Navicula radiosafallax* Lange-Bert.	96
Frustulia saxonica Rabenh.	97	*Navicula rhynchocephala* Kütz.	96
Gomphonema angustum Agardh	100	*Navicula saprophila* Lange-Bert. & Bonik	91
Gomphonema constrictum Ehrenb.	91	*Navicula seminulum* Grunow	97
Gomphonema gracile Ehrenb.	94	*Eolimna subminuscula* (Manguin) Moser, Lange-Bert. & Metzeltin	96
Gomphonema minutum (Agardh) Agardh	93	*Navicula tripunctata* (O.F.Müller) Bory de Saint-Vincent	97
Gyrosigma acuminatum (Kütz.) Rabenh.	98	*Nitzschia communata* Grunow	100
Navicula cincta (Ehrenb.) Grunow	97	*Nitzschia dissipata* (Hantzsch) Grunow	99
Navicula gregaria Donkin	90	*Nitzschia draveillensis* Coste & Ricard	100
Navicula joubaudii Germain	99	*Nitzschia frustulum* (Kütz.) Grunow	94
Navicula radiosafallax Lange-Bert.	97	*Nitzschia linearis* (Agardh) W. Smith	92
Navicula suchlandtii Hustedt	100	*Nitzschia pumilla* Hustedt	100
Navicula tridentula Kasske	95	*Reimeria sinuata* v. *delognei* (Grunow) Lange-Bert.	98
Navicula wildii Lange-Bert.	100	*Thalassosira pseudonana* Hasle & Heimdal	97
Nitzschia fonticola Grunow	97		
Nitzschia prolongata Hustedt	100		
Nitzschia pseudofonticola Hustedt	100		
Nitzschia recta Hantzsch	93		
Pinnularia brauniana (Grunow) Mills	93		
Pinnularia legumen Ehrenb.	92		
Surirella brebissonii Krammer & Lange-Bert.	100		

Table 4 – A. MRPP of the 13 different pond classes Sajaloli & Dutilleul (2001). Light gray blocks having non– significant p values. Dark gray blocks had the greatest separation. B. MRPP of 6 new classes based on the results of the MRPP of 13 pond classes

A – 13 pond classes	*Decidous forests*	*Pine forests*	*Coppices*	*Edges of forests*	*Forestry areas*	*Health lands – moors*	*Freeways – toll ways*	*Country roads*	*Prairies*	*Fields*	*Exterior to buildings*	*Edges of villages*	*Interior villages*	
Decidous forests		– 2.93	– 7.16	–1.27	– 3.50	– 5.34	– 9.47	– 2.04	**– 11.64**	**– 16.81**	**– 14.46**	**– 13.83**	**– 17.17**	T values
Pine forests	0.012		– 3.28	– 1.55	– 1.90	– 3.06	**– 10.90**	– 2.47	**15.46**	**– 18.99**	**– 14.34**	**– 13.96**	**– 16.28**	
Coppices	0.001	0.008		– 6.68	– 2.91	– 3.42	**– 16.15**	– 6.40	**– 18.49**	**– 21.73**	**– 19.52**	**– 17.81**	**– 21.00**	
Edges of forests	**0.108**	**0.078**	0.001		– 2.45	– 0.86	– 1.98	0.13	– 6.64	– 9.17	– 4.44	– 3.49	– 5.52	
Forestry areas	0.006	0.046	0.013	0.023		– 2.67	– 9.47	– 1.70	**– 13.66**	**– 18.24**	**– 13.90**	**– 12.41**	**– 14.61**	
Health lands/moors	0.001	0.010	0.008	**0.171**	0.020		– 6.98	– 1.06	**– 12.06**	**– 17.96**	**– 10.93**	**– 9.35**	**– 10.13**	
Freeways – toll ways	0.001	0.001	0.001	0.048	0.001	0.001		– 5.35	– 2.53	– 6.91	– 1.46	– 1.92	– 1.54	
Country roads	0.043	0.023	0.001	**0.482**	**0.065**	**0.135**	0.001		– 9.08	**– 15.36**	– 8.85	– 8.59	– 9.91	
Prairies	0.001	0.001	0.001	0.001	0.001	0.001	0.024	0.001		– 2.02	– 2.68	– 2.77	– 4.66	
Fields	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.040		– 6.85	– 7.20	**– 10.78**	
Exterior to buildings	0.001	0.001	0.001	0.002	0.001	0.001	**0.087**	0.001	0.018	0.000		– 0.26	– 1.21	
Edges of villages	0.001	0.001	0.001	0.006	0.001	0.001	**0.050**	0.001	0.012	0.001	**0.340**		0.54	
Interior villages	0.001	0.001	0.001	0.001	0.001	0.001	**0.079**	0.001	0.001	0.001	**0.117**	**0.667**		
	P values													

B – 6 pond classes	*Forests*	*Coppices*	*Health lands/ moors*	*Roads*	*Prairies/ Fields*	*Villages*	
Forests		– 6.98	– 4.31	– 7.25	– 35.41	– 41.44	T values
Coppices	0.001		– 3.42	– 14.45	– 29.86	– 32.08	
Health lands/moors	0.001	0.007		– 3.92	– 22.73	– 16.58	
Roads	0.001	0.001	0.003		– 14.13	– 9.18	
Prairies/Fields	0.001	0.001	0.001	0.001		– 12.55	
Villages	0.001	0.001	0.001	0.001	– 12.55		
	P values						

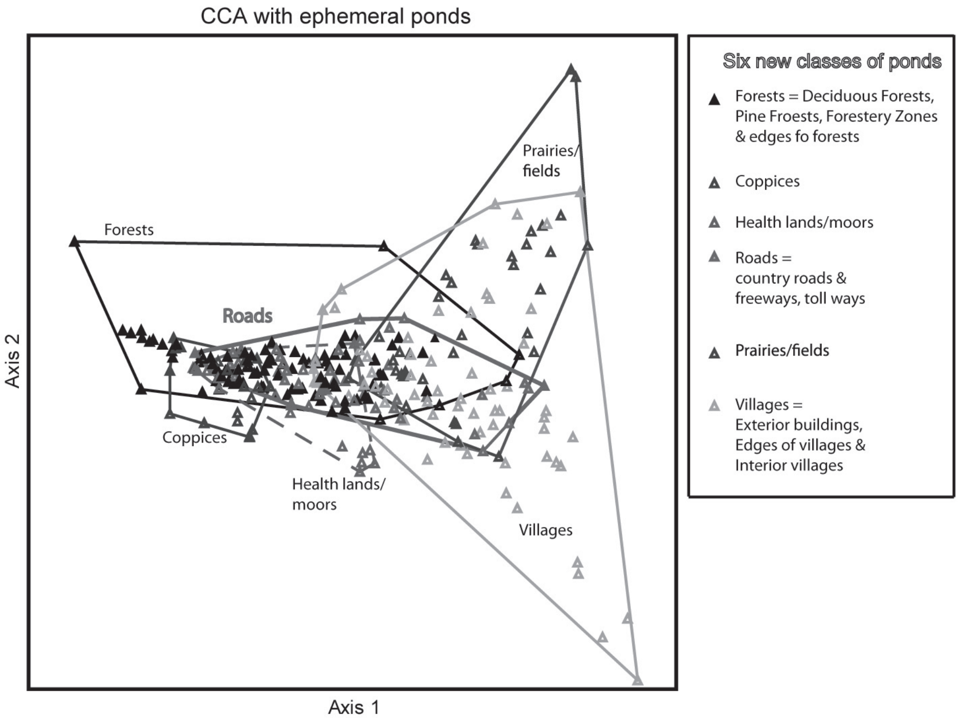

Fig. 1. Canonical correspondence analyses of the ephemeral pond samples showing the relative position of the six pond classes. The road class, outlined in bold lines, was distributed over several groups.

to that class (Table 5). However, only one species had an indicator value > 50%, *Planothidium lanceolatum* (Bréb.) Lange-Bert. for the prairies/fields class (58.1%, p = 0.03).

A MRPP of the 12 growth categories (Table 6) from the ponds had T = –10.14 and p = 0.0001. The *Sphagnum* was statistically separated from almost all the other groups with the exception of plankton, with a T values that varied from –4.21 (submerged grasses) to –10.9 (dead submerged branches and stems); mud was separated from submerged living roots, rocks, aquatic plant – algae, submerged mosses, dead submerged branches – stems, *Sphagnum*, and submerged dead leaves. Submerged living roots and rocks differed from each other and from aquatic plants – algae, submerged grasses, submerged mosses, dead submerged branches, stems and leaves. No significant difference was observed between mud, submerged stems and plankton. Nevertheless, IS analysis suggests that several species occurred in > 70 % of the samples for each growth categories (Table 7), with the exception of mosses with zero species that have an environmental preference to mosses. Mud and submerged stems had the most species (respectively eight and four). Examining a CCA plot, it became evident that *Sphagnum*, live stems, walls, roots, plankton, rocks, mud and maybe submerged grasses had limited distribution (Fig 2A), while analysis of dead leaves, mosses, aquatic plants – algae, dead branches – stems and maybe grasses seem to have a large coverage (Fig 2B).

Table 5. List of species found in > 90 % of the land use class in the different six pond classes

Forests	%
Achnanthes rupestroides Hohn	100
Cyclostephanos dubius (Fricke) Round	100
Fragilaria crotonensis Kitton	100
Gomphonema angustum Agardh	100
Nitzschia draveillensis Coste & Ricard	100
Cyclotella pseudostelligera Hustedt	97
Navicula menisculus Schumann	97
Reimeria sinuata v. delognei (Grunow) Lange-Bert.	96
Pseudostaurosira brevistriata (Pant.) Edlund	95
Amphora pediculus (Kütz.) Grunow	92
Aulacoseira granulata (Ehrenb.) Simonsen	92
Nitzschia dissipata (Hantzsch) Grunow	91

Coppices	%
Fragilaria bidens Heiberg	100
Pinnularia brevicostata Cleve	94

Health lands – moors	%
Nitzschia pseudofonticola Hustedt	100
Navicula perminuta Grunow	96
Amphipleura pellucida (Kütz.) Kütz.	93

Roads	%
Brachysira neoexilis Lange-Bert.	100
Eunotia steineckii Petersen	97
Stenopterobia curvula (W.Smith) Krammer	95
Eunotia romboidea Hustedt	91

Field – prairies	%
Navicula wildii Lange-Bert.	100
Cymbella cesati (Rabenh.) Grunow	97
Brachysira brebissonii R.Ross	92

Villages	%
Nitzschia prolongata Hustedt	100
Surirella brebissonii Krammer & Lange-Bert	100
Navicula cincta (Ehrenb.) Grunow	99
Craticula accomoda (Hustedt) D.G.Mann	94
Navicula joubaudii Germain	94
Eolimna subminuscula (Manguin) Moser, Lange-Bert. & Metzeltin	92
Craticula halophyla (Grunow) D.G.Mann	90

Water chemistry

The ephemeral pond samples were removed as certain sites were missing some of the chemical data, and multi-collinearity of the chemical factors was determined using R. pH, log conductivity and surface area explained some of the difference between the two regions (Fig. 3). The pH gradient was associated with axis 1 (2.8 % variance), with highest values on the left of the ordination. Conductivity and surface area increased in the upper half of the graph. Ponds from the Orléans – Sologne region clustered in the upper left quadrant (higher conductivity) and upper right quadrant (larger surface area). There appears to be a higher concentration of higher pH ponds in the Beauce region than in the Orléans – Sologne region.

CCA conducted on each region separately showed that the variables explained the higher percent of variance than the two regions together. For the Orléans – Sologne region (axis 1, 3.7% variance), log conductivity and SiO_2 were the two main factors (Fig. 4). The highest log conductivity registered on the left half of the graph and it separated the different six pond classes with prairie/field ponds having the highest conductivity, following by areas of human occupation, then

Table 6. MRPP results of the different diatom growth supports. Light grey having not significant difference. Dark grey having insignificant p values

	Mud	Walls, plastic, neutral supports	submerged living roots	*Rocks*	Aquatic plants – algae	*Submerged grasses*	*Submerged stems*	*Plankton*	*Submerged mosses*	*Dead branches and stems*	*Sphaignum*	*Submerged dead leaves*	
Mud		**0.05**	– 4.02	– 2.97	– 5.52	– 5.34	**– 1.2**	**1.16**	– 4.71	– 4.09	– 6.64	– 4.21	T value
Walls, plastic, neutral supports	**0.4391**		**1.04**	**1.4**	**– 1.39**	**– 1.52**	**0.96**	**1.35**	**– 1.59**	**– 0.29**	– 4.73	**– 1.29**	
Submerged living roots	0.0022	**0.8578**		**– 1.65**	– 5.68	4.14	**– 0.23**	**0.28**	– 6.01	– 2.9	7.55	– 6.00	
Rocks	0.0112	**0.9429**	**0.0566**		– 6.73	– 6	**– 0.39**	**0.69**	– 6.11	– 4.04	– 7.34	– 6.11	
Aquatic plants – algae	0.0001	**0.0922**	0.0001	0.0001		**– 0.19**	**– 1.05**	**0.68**	**– 0.17**	– 3.77	– 7.61	– 6.04	
Submerged grasses	0.0002	**0.0778**	0.0021	0.0001	**0.3577**		**– 1.42**	**0.95**	**– 1.17**	**– 1.9**	– 4.10	**– 1.25**	
Submerged stems	**0.1173**	**0.8396**	**0.3607**	**0.3102**	**0.1432**	**0.0902**		**1.27**	**– 1.81**	**0.52**	– 7.08	**– 1.46**	
Plankton	**0.9130**	**1.0000**	**0.5749**	**0.5029**	**0.7417**	**0.8302**	**0.9113**		**0.6**	**0.64**	**0.80**	**0.52**	
Submerged mosses	0.0004	**0.0697**	0.0001	0.0001	**0.3645**	**0.1230**	**0.0534**	**0.7028**		**– 1.91**	– 5.85	**– 1.19**	
Dead branches and stems	0.0018	**0.3314**	0.0119	0.0021	0.0037	**0.0494**	**0.6532**	**0.7178**	0.0484		– 10.9	– 4.38	
Sphaignum	0.0003	0.001	0.0002	0.0002	0.0001	**0.0024**	0.0001	**0.5245**	0.0002	0.0001		– 6.12	
Submerged dead leaves	0.0016	**0.1053**	0.0001	0.0001	0.0001	**0.1108**	**0.0861**	**0.6380**	**0.1204**	0.0017	0.0001		
	P value												

Table 7. List of species that grow on a particular support in greater than 75%

Mud	%
Amphora ovalis (Kütz.) Kütz.	89
Craticula accomoda (Hustedt) D.G. Mann	97
Pseudostaurosira brevistriata (Pant.) Edlund	98
Melosira varians Agardh	89
Nitzschia hungarica Grunow	86
Pinnularia brebissonii Kütz.	76
Rhoicosphenia abbreviata	81
Surirella brebissonii Krammer & Lange-Bert	99

Submerged stems	%
Amphipleura pellucida (Kütz.) Kütz.	89
Cymbella cesati (Rabenh.) Grunow	77
Nitzschia acicularis (Kütz.) W. Smith	82
Nitzschia communata Grunow	95

Dead branches – stems	%
Achnanthes rupestoides Hohn	100
Fragilaria crotonensis Kitton	100
Navicula seminulum Grunow	93

Submerged living roots	%
Navicula suchlandtii Hustedt	85
Navicula wildii Lange-Bert.	93

Rocks	%
Ulnaria acus (Kütz.) M.Aboal	90

Plankton	%
Nitzschia draveillensis Coste & Ricard	95

Dead leaves	%
Brachysira neoexilis Lange-Bert.	100

Submerged grasses	%
Nitzschia pseudofonticola Hustedt	90
Pinnularia brevicostata Cleve	77

Sphagnum	%
Eunotia septentrionalis Oestrup	75
	86

Submerged plants – algae	%
Fragilaria bidens Heiberg	100

Wall, plastic, neutral supports	%
Navicula menisculus Schumann	77

Mosses	%
None	

forest, road and health lands/moors mixed together while coppices had the least. There was a wider range of SiO_2 content within the different classes with again coppices having the least variation. As for the Beauce region, pH and K were the two main factors (Fig. 5). Axis 1 (4.5% variance) corresponded almost to the pH gradient, with the right half to the graph having the highest pH with alkaline ponds and the left half with the acid ponds. The highest K rich ponds were situated in the lower right quadrant while the least were in the upper left quadrant. Forest, health lands/moors and coppices had the lowest K values while roads, fields and villages had the highest.

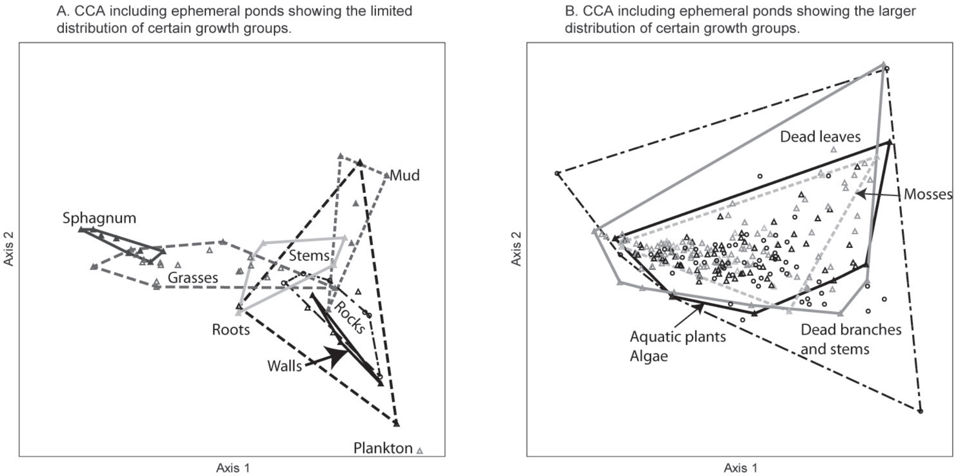

Fig. 2. Canonical correspondence analyses of the 12 growth support groups including the ephemeral ponds. A. The growth supports with limited distribution live stems, walls, roots, rocks, mud and plankton. B. Growth support with larger distribution are dead leaves, mosses, aquatic plants – algae, and dead branches and stems.

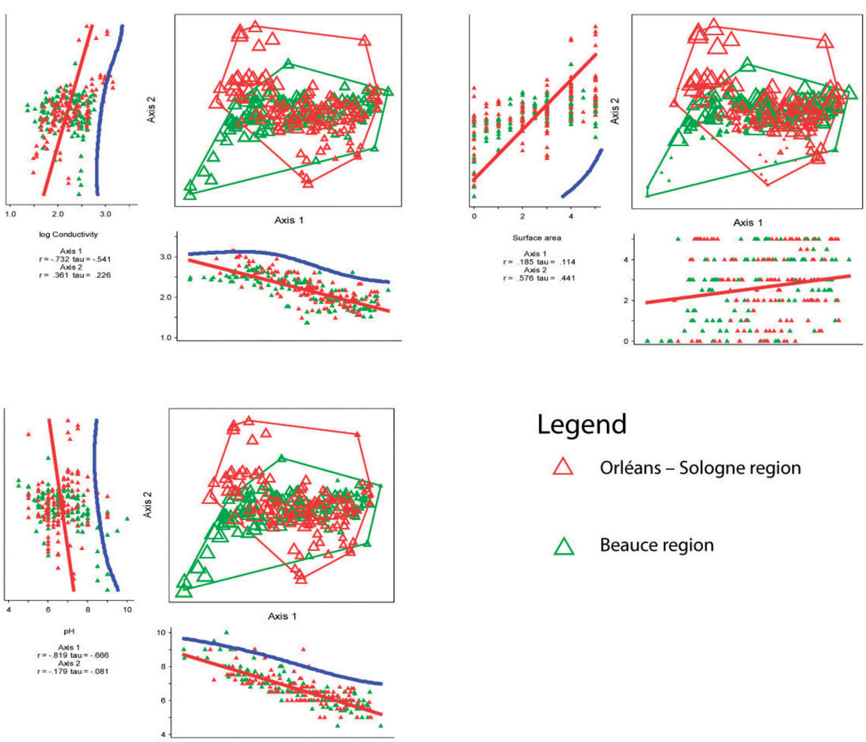

Fig. 3. Canonical correspondence analyses of three factors, pH, conductivity and surface area determined by R that separated the two regions, Beauce and Orléans – Sologne.

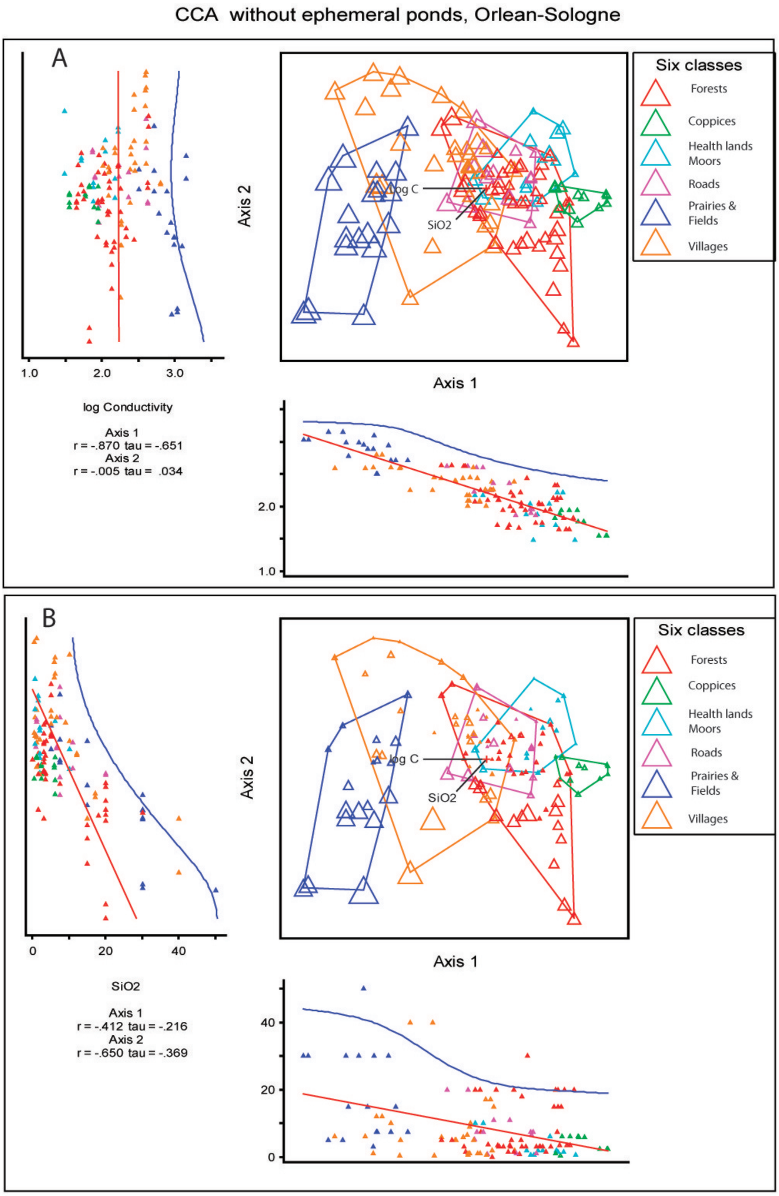

Fig. 4. Canonical correspondence analyses of two factors, SiO_2 and log conductivity (log C), determined by R that separated the six new pond classes for the Orléans – Sologne region.

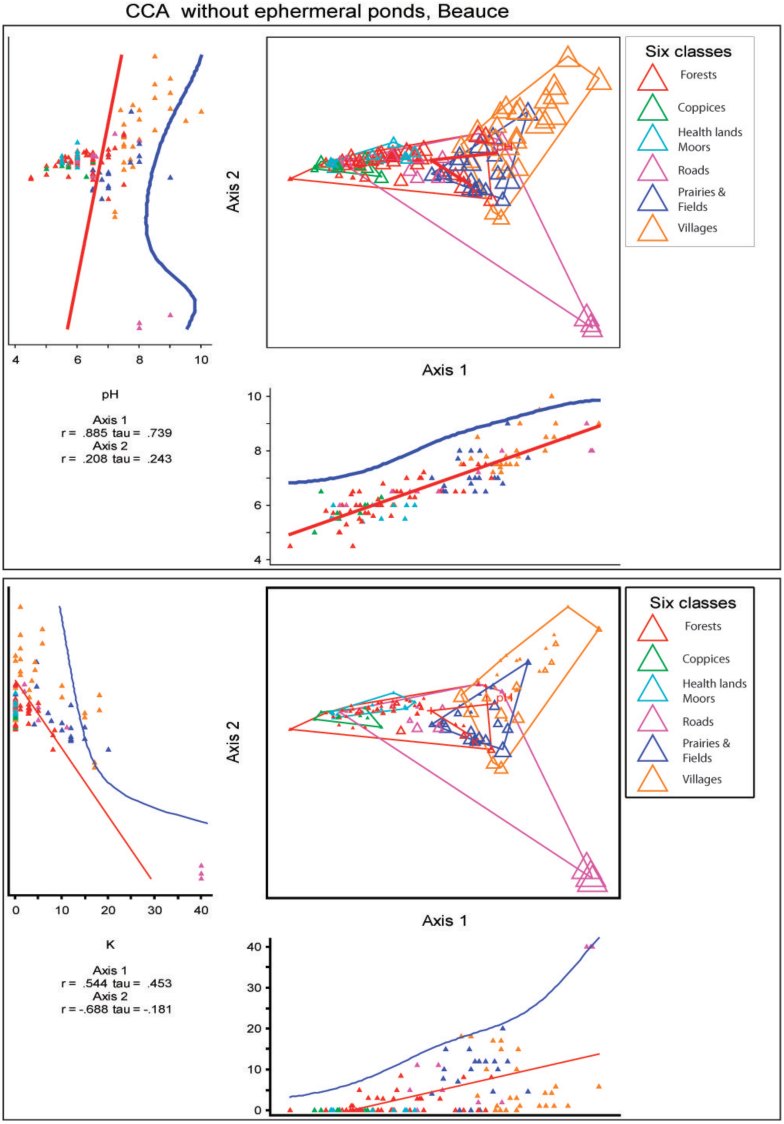

Fig. 5. Canonical correspondence analyses of two factors, pH and K, determined by R that separated the six new pond classes for the Beauce region.

DISCUSSION

Very little research has been done on artificial ponds (Bertrand *et al.*, 2013, 2014a, b), but they could be of ecological importance in the future as zone for storing precipitation for dry periods, a zone for water retention during heavy rainfall, and a source of water for recharging the water table, besides providing farmer with usable water. Each region would have different uses for artificial ponds that could benefit their region. Ponds are being created in areas of extraction but they could also be developed in hilly areas with the blocking of small valleys that are not used for agricultural prepossess, a common practice in the Midwest United States.

In 2009, Poulíčková *et al*. examined the epipelic diatoms in artificial fish ponds and found strong relationships between species composition and environmental factors. They found that niche-based control rather than the effects of dispersal limitations was important. Špačková *et al.* (2009) found a seasonal succession of epipelic algae in a mesotrophic pond; diatoms were dominant in the spring and autumn with planktonic diatoms deposited in summer.

Bertrand *et al.* (2013) found that the eight genera (*Eunotia* Ehrenb., *Navicula* Bory, *Achnanthes* Bory, *Nitzschia* Hassall, *Gomphonema* C. Agardh, *Fragilaria* Lyngbye, *Cymbella* C. Agardh and *Pinnularia* Ehrenb.) contained the highest abundances and relative frequencies in ponds, while dead leaves, aquatic plants and dead branches were the most frequently found substrates, respectively 32.1%, 24.3% and 19.0%. Nevertheless, our results determined that every growth category had at least one or more species that showed a preference for that category in > 70% of the samples, with exception of mosses (none). As Williams *et al.* (2004) noted ponds have exceptionally high biodiversity and according to Céréghino *et al.* (2008), ponds are often considered to be "hot spot" of biodiversity. They could be areas where rare species or species on the edge of extinction may take refuge. These unique species would most likely not contribute to a water quality index. However, if the objective of the program is not analyzing water quality but biodiversity or rare species, the IS and CCA results should be taken into consideration as to what growth supports should be sampled.

In Bertrand *et al*. (2014a), no difference was found in the diatom population between the two regions, but our CCA results showed that pH, conductivity and surface area separated the two regions and each region had different chemical factors effecting the diatom populations (Orléans – Sologne, log conductivity & SiO_2; Beauce, pH & K). Their factorial analysis of correspondences found a separation between three land use classes (forest, urban and villages) but their principal component analysis found only a separation of the freeways/toll ways classes from the other land use classes. However, our MRPP which tests the hypothesis of no difference between two groups determined five classes of land use (forests, coppices, heath lands/moors, roads, prairie/fields and villages) based on the diatom populations, but the roads class needed to be reexamined and classified according to the surrounding land use. Moreover, logical divisions were created allowing for (i) the determination of diatom land use preferences and (ii) a comparison of regional land use effects. These diatom land use preferences could be of value in paleolimnological studies with the purpose of determining land use changes.

Beauger *et al.* (2013) also found in a study of six oxbow lakes that certain species were associated to land use. When comparing regional land use effects for the Beauce region, the samples from coppices and heath lands/moors clustered with the forest samples. These land use classes have large amounts of organic acid added to the ponds which decay creating humic acid, thereby lowering the pH while the other land use classes (roads, prairies-fields and villages) may have less organic input and therefore could have more acid neutralization by calcareous rocks decomposition. When comparing the two regions, pine forests are known to acidify the soil but this effect seems to be less significant in the Beauce than in the Orléans – Sologne regions where log conductivity showed collinearity with pH, with a greater dispersion of the samples from forest, coppices and heath lands/moors. Prairies/fields and land surfaces in and around villages had higher percentage of the K in the Beauce region, most likely related to fertilization, but the highest were registered for several road sites. This could be the effect of salting the roads in the winter. In the Orléans – Sologne regions, the highest conductivities were related to prairies/fields and villages. As seen in the Beauce region, this is most likely related to fertilization. However, the Orléans – Sologne regions are composed many of sands and clays which have less buffering capacity along with the fact that sandy soils have fewer nutrients available for plant growth. Therefore, it appears there is more fertilization occurring in this region. Another effect of less buffering could be the dissolution of the sand and clay liberating more SiO_2 into the system. Prairies/ fields, villages and forests samples showed a gradient from large amounts of SiO_2 while coppices and health lands/moors had limited amounts.

Beauger *et al.* (2013) also found that certain species have preferences for certain growth supports such as pebbles and cobbles, macrophytes and mud-organic matter, and sand and mud-organic matter. Analysis of diatom growth supports showed that stems, walls, roots, plankton, rocks, mud and maybe submerged grasses had limited distribution within the ponds and therefore should not be used in a protocol for sampling diatoms in artificial ponds. If a protocol was to be adopted, it appears that the best approach for recovering maximum amount of information and optimizing the evaluation of the ecology would be to collect a composite sample from the following substrates: (i) aquatic plants – algae, (ii) mosses, and (iii) submerged dead leaves, branches and stems, and (iv), even submerged grasses.

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