Vol. 80: 61–75, 2017
https://doi.org/10.3354/ame01836

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online August 21

Algal communities of spring-associated limestone habitats

Manuela Trobej, Jean-Pierre Bednar, Johann Waringer, Michael Schagerl*

Department of Limnology and Oceanography, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

ABSTRACT: Travertine-depositing headwaters are characterized by unique hydrochemical and geomorphological features that are reflected in specialized assemblages. We studied microphytobenthos of 14 spring-associated limestone (SAL) sites in 2 different geologic zones of Austria twice in a year to check if any spatial or temporal variations could be recognized. Multivariate statistics (cluster analysis, non-metric multidimensional scaling) did not reveal temporal differences. However, a geographical pattern was obvious, with sites of western Austria clearly separated from other locations. The 7 resulting groups are characterized by 22 indicator species such as *Brachysira calcicola* ssp. *pfisteri* and *Cymbopleura subaequalis*. In addition to the diatom taxa typical of SAL habitats (*Delicata delicatula*, *Gomphonema lateripunctatum*), the rare desmid *Oocardium stratum* was also found at 7 sites, including new discoveries at 3 sites. Canonical correspondence analysis showed that input variables anthropogenic disturbance, total phosphorus, sky openness, chloride concentration and calcium carbonate precipitation rate significantly influence algal species distribution. Some of these variables reflect anthropogenic impact and therefore suggest limited stability of SAL communities against pollution.

KEY WORDS: Spring · Headwater · Travertine · Tufa · Periphyton · Algae · *Oocardium*

INTRODUCTION

Springs are groundwater-dependent ecosystems linking lentic, lotic and semi-terrestrial habitats (Kløve et al. 2011). Springs and headwater streams provide several ecosystem services, such as drinking water supply and recreational opportunities. Compared to other aquatic ecosystems, springs and adjacent headstreams have a very high biodiversity because they offer various substrata for colonization and high food availability (Stanford et al. 1994, Cantonati et al. 2006). They harbour both groundwater and surface water taxa, and both terrestrial and wetland species, all of which take advantage of the groundwater buffering capacity against dryness and temperature fluctuations (Kløve et al. 2011).

There exist various types and classifications of springs (Kresic 2010). Travertine springs (in Central Europe sometimes called tufa springs) are one type of spring listed in these classification systems. Travertine is the result of limestone precipitation caused by CO_2 loss from water into the atmosphere. Turbulences and the active removal of CO_2 through photosynthesis accelerate this process, which causes a shift from inorganic processes at the spring exit to biogenic processes downstream (Merz & Zankle 1991). 'Meteogene travertine' is used to describe all deposits where CO_2 originates from soil and root respiration, and thus was originally fixed from the atmosphere (Pentecost 1993, Pentecost & Viles 1994). Sanders et al. (2011) introduced another term for meteogene travertine, which is spring-associated limestone (SAL).

In SAL habitats, diatoms, cyanobacteria, heterotrophic bacteria and microscopic chlorophytes are essential for biofilm development; they moreover provide crystallization nuclei for carbonate precipitation (Cantonati et al. 2006, Golubić et al. 2008). Cantonati et al. (2012b) observed that diatoms and cyanobacteria were the most common algal groups in SAL springs and that pH, conductivity, inorganic

*Corresponding author: michael.schagerl@univie.ac.at

nitrogen, substrate particle size and irradiance supply are key parameters for species occurrence.

In Europe, SAL springs are the only type that is registered in the European Habitat Directive (European Union 1992), where they are classified as 7220 Petrifying springs with tufa formation (*Cratoneurion*); other spring types and small headwaters (catchment <10 km^2) are not considered in the Water Framework Directive (European Union 2000). Although springs are central elements of the water cycle, studies on variables structuring spring biocoenoses are scarce; such research is however essential for keeping these highly threatened habitats intact and for protecting them (Weigand 1998, Cantonati et al. 2006, Ilmonen et al. 2012).

This study was performed to check for any spatial and temporal variations in SAL habitats located across different geologic zones in Austria. Permanent springs are stable biotopes in terms of their physicochemistry. Moreover, the unique conditions provided in SAL habitats suggests that, independent of the geographic origin, a similar community composition of specialists is found, which are able to cope with the ongoing deposition of calcium carbonate. We therefore expected only marginal temporal and spatial variations of the phytobenthos composition.

Species composition was correlated to selected environmental variables to gain insight into key variables structuring community composition with particular paid to the desmid *Oocardium stratum* Nägeli. Although desmid diversity and abundance tends to be greatest in acidic habitats with low ion concentrations, this taxon is restricted to SAL habitats and adjacent headstreams with active carbonate deposition (Wallner 1933, 1934, Sanders & Rott 2009). *Oocardium* is distributed worldwide, but it occurs in very specific and widely scattered locations in Croatia (Matoničkin & Pavletić 1961), South of Bavaria (Swabian Alb) (Wallner 1933), USA (Mathews et al. 1965), the British Isles (West & West 1901, Pentecost 1981), Ireland (Adams 1908) and Belgium (van Oye & Hubert 1937). The rare occurrence was already scrutinized by Pfiester (1976), who mentioned that SAL habitats are seldom visited by phycologists, therefore *Oocardium* might have been overlooked in the past. To test Pfiester's assumption, we visited locations suitable for *Oocardium* growth and we expected new discoveries especially at sites with increased carbonate precipitation. We assumed that *O. stratum* is dependent on free CO_2 and that it has some advantage over other taxa because of its unique growth strategy, which is construction of a characteristic calcareous tube system.

MATERIALS AND METHODS

Study sites

We studied 14 travertine springs and adjacent headwaters of Stream Order 1 (Strahler 1957, Vannote et al. 1980), which showed active travertine formation (Table 1 gives the site names and abbreviations used hereafter). They are located in 2 different geologic zones of Austria: Limestone Alps and Molasse Zone (Fig. 1).

In situ measurements

The sites were sampled twice in 2014, once in the period between late May to July, and once in September, along the carbonate deposition stretch (corresponding phenological growing seasons are full spring to early summer and autumn). A sketch was

Table 1. Names, abbreviations (ID) and coordinates of sampling sites used for the study of algal communities of spring-associated limestone (SAL) sites in Austria

Site	ID	Coordinates
Woellersdorf, Lower Austria	Woe	N 47.8567, E 16.1364
Maiszinken at Lake Lunz (top), Lower Austria	Lut	N 47.8613, E 15.0675
Maiszinken at Lake Lunz (bottom), Lower Austria	Lud	N 47.8599, E 15.0666
Side arm of the Poellerbach in Alland, Lower Austria	Poe	N 48.0557, E 16.0259
Almassyschloessl next to Schloeglmuehl Gloggnitz, Lower Austria	Alm	N 47.6998, E 15.9043
Alpenzoo Innsbruck, Tyrol	Zoo	N 47.2831, E 11.3990
Hochtalalm, Tyrol	Hoc	N 47.5491, E 11.8881
Lingenau, Vorarlberg	Lin	N 47.4440, E 9.9072
Preinmuehle next to Schwarzau/ Preintal, Lower Austria	Pre	N 47.7946, E 15.6601
Maria Neustift, Upper Austria	Mar	N 47.9308, E 14.6108
Dandlgraben next to Maria Neustift Losenstein, Upper Austria	Dan	N 47.9305, E 14.4513
Lappenbach next to Stein Drauntal, Carinthia	Lap	N 46.7271, E 13.0407
Teschengraben, Lower Austria	Tes	N 47.9263, E 14.8006
Edlbach next to Spital am Phyrn, Upper Austria	Edl	N 47.6732, E 14.3510

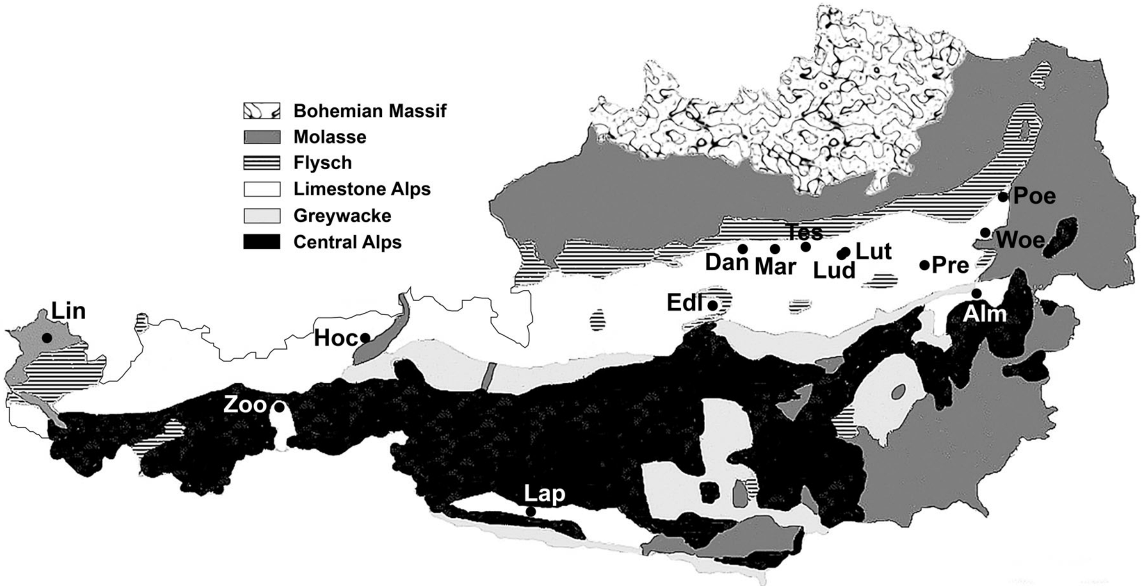

Fig. 1. Locations of sampling sites used for the study of algal communities of spring-associated limestone (SAL) habitats in Austria, showing geological zones. For full names of sites corresponding to the abbreviations refer to Table 1

drawn of this stretch and an estimate made of the percent cover of the 3 predominant lithological substrata: travertine surfaces, gravel (2 to 20 mm diameter) and sand (0.2 to 2 mm diameter), which together made up 100% of the studied area. Sand was intermixed with mud, which is referred to as psammopelal. All measurements were performed in triplicate except carbonate precipitation rate, slope and anthropogenic disturbance (see below). Specific conductivity, water temperature (WTW LF197i), pH (WTW pH 330i/Metrohm combined glass electrode 6.0253.100) and O_2 concentration (WTW Oxy 197i) were measured on site. Free carbon dioxide and bicarbonate (HCO_3^-) were titrated on site with unfiltered water with HCl (0.1 N) and NaOH (0.01 N) to the pH endpoints 3.4 and 8.2, respectively, with calculations performed according to Hütter (1994). To estimate sky openness as a proxy for irradiance supply, photos were first taken with a levelled camera positioned towards the sky and equipped with a fisheye lens (Nikon Coolpix 4500; Nikon fisheye converter FC-E8 0.21× objective). The calculation of sky openness was done with the gap light analyzer (GLA) version 2.0) (Frazer et al. 1999).

Nails with washers were mounted in spring and collected in autumn to estimate the total calcium carbonate precipitation rate (CCP) including both biotic and abiotic processes. After exposure in the field, the carbonate layer was measured under a binocular microscope (Zeiss SteREO Lumar.V12) as follows: the washers were vertically clamped with a paper clip and pictures were taken. Then travertine thickness was measured with the Zeiss ZEN 2011 (blue edition) software.

Slopes of headwaters were measured with a flexible tube water level (tube length 20 m), water velocity was estimated with methylene blue by adding drop by drop and measuring the time taken by drops to reach the end of a defined stretch (Pomeisl 1953). Discharge was calculated from the width, depth and water velocity. Anthropogenic disturbance was categorized based on the surrounding of the headwater on an ordinal scale, where 0 = undisturbed, i.e. difficult to access, no channelling, no human activities; 1 = weak disturbance, e.g. hiking trails nearby, but no direct access; 2 = little disturbed, e.g. roads in the close vicinity of the spring, at most very moderate agricultural land use in the surrounding area; 3 = moderately disturbed, e.g. nearby agricultural land use or deforestation, 4 = strongly disturbed, e.g. stream regulation (presence of control structures) and direct human impact; and 5 = highly disturbed, e.g. total water obstruction for drinking water supply.

Chemical analysis

Water samples (unfiltered and filtered) were taken in triplicate and stored cold and dark until chemical analysis in the laboratory. The water was filtered on site with syringe-filter holders (Whatman glass microfiber filters, 47 mm diameter). Calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), chloride (Cl^-), potassium (K^+), sulphate (SO_4^{2-}) and nitrate (NO_3^-) were analysed by ion chromatography (Metrohm Compact IC 761, Suppressor-modul MSM, Metrohm 853 CO2-Suppressor, Metrohm IC Filtration Sample Processor 788; anions Metrohm Metrosep A Supp 5 150 × 4.0 mm ID with suppression; cations Metrohm Metrosep C2 150 × 4.0 mm ID) (OENORM EN ISO 10304-1:2012-06-01, OENORM EN ISO 14911:1999-11-01). Ammonium (NH_4^+), nitrite (NO_2^-), phosphate (PO_4^{3-}) and total phosphorous (TP) were analysed spectrophotometrically (Hach-Lange DR 2800) (DIN EN 26777:1993-04, OENORM EN ISO 6878:2004-09-01, OENORM ISO 7150-1:1987-12-01). Dissolved organic carbon was analysed according to the UV/difference method (Sievers 5310 C) (DIN EN 1484). All the parameters except for TP were analysed with the filtered water. The Langelier Saturation Index (LSI) was calculated with the Lenntech online calculator (www.lenntech.com/calculators/langelier/index/langelier.htm); an index value greater than zero indicates supersaturation of $Ca(HCO_3)_2$ and high probability of carbonate precipitation, an index value less than zero indicates supersaturation of H_2CO_3 and high probability of corrosion.

Biological samples

Biofilm samples were taken from each of the 3 main microhabitats (i.e. travertine, gravel and sand) with a self-made sampling device: from a plastic bottle with a wide opening, the bottom was cut and the bottleneck placed towards the substratum to define an exact sampling area (Douglas 1958). For each microhabitat, 3 subsamples were taken within the section and merged to a single sample. For the travertine microhabitat, the bottleneck was placed on the surface and the outline was drawn with a knife. Then the knife was used to scrape off the defined area. Gravel was collected inside the bottleneck area and afterwards brushed for sampling the biofilm. For sand, a syringe was connected to a tube, which was placed inside the bottleneck of the sampling device to exhaust the area. Half of the material was used for studying live material; the other part was first fixed with ethanol for DNA extraction of heterotrophic bacteria (no data available), and then preserved with Pfeiffer's mixture (Esser 1982) for relative abundance estimations. Whenever possible, algal groups other than diatoms were identified as live material within a few days after collection to omit artefacts caused by preservatives (some key features might be lost because of fixation). For diatom identification, both wet combustion (HCl, HNO_3 + H_2SO_4) and dry combustion according to protocols of Krammer & Lange-Bertalot (2007b) were implemented to remove organic substances. Wet combustion results in very clean diatoms without any carbonates, but because of the strong acids, frustules disintegrate. Dry combustion provides information on the whole frustules. The slides were mounted in Naphrax after placing them in 5% HCl followed by rinsing with MilliQ water to remove carbonate layers which would otherwise disturb the microscopical studies. Identification was done with a compound microscope (Zeiss Axio Imager.M1, AxioCam MRc5, AxioCam MRm). Identification was done to the lowest level possible with Zeiss Planapo-objectives 40×, 63× and 100×. Relative abundance estimations were done by screening the whole cover glass area, abundance was estimated on a modified DAFOR scale with values ranging from 1 = rare (<2%), 2 = occasional (2 to <10%), 3 = frequent (10 to <25%, 4 = abundant (25 to <50%) to 5 = dominant (≥50%) (Kent & Coker 1992). Compared to absolute counts, which often display a highly skewed distribution, such coding already implies a transformation similar to a log-transformation, which is suggested to improve normality and homogeneity of variances among groups (McCune & Grace 2002). For identification of algae, the following keys were used: Komárek & Anagnostidis (2005, 2013), Krammer (1997), Krammer & Lange-Bertalot (2004, 2007a,b, 2008), Hofmann et al. (2011), Rieth (1980) and Eloranta et al. (2011). Bryophytes were identified by Dr. Robert Krisai and followed the key of Frahm & Frey (1992).

Statistics

Statistics was carried out with the R package (Version 3.1.3, R Development Core Team, Vienna) using the packages vegan, MASS, xlsx, gclus, pvclust and labdsv (Borcard et al. 2011). First, a dissimilarity matrix with the taxa frequencies of sites and dates was calculated using the Bray-Curtis index (functions: vegdist and hclust) to evaluate spatial and/or temporal similarities among the biological data. The best agglomeration method (UPGMA) was selected for cluster analysis (function: cophentic). A mantel test was carried out to find the optimum number of clusters. Afterwards, an analysis of similarities (function: anosim) was used to test for significant pruning of clusters. Non-metric multidimensional scaling (nMDS) was calculated 4 times with the metaMDS function, using the dissimilarity matrix with 2 dimensions. The number of random starts was 100, the function use for the MDS was monoMDS, the maximum number of iterations was set to 2000 and the start searched for the best previous solution. In addition, an indicator species analysis (Dufrêne & Legendre 1997) was calculated with the indval function. A species is regarded as highly indicative of a particular site when it is mainly found in only that site and is present in a large number of its sample units (maximum indicator value = 100%). To link patterns of species occurrence with environmental variables, a direct gradient analysis was performed. First, a detrended correspondence analysis was carried out; the gradient lengths (Axis 1: SD = 2.68, Axis 2: SD = 2.79) suggested that constrained correspondence analysis (CCA) was suitable (ter Braak & Prentice 1988), which was then carried out using relative abundance species data and *z*-standardized environmental data. An automatic forward selection was carried out with the ordistep function. In a second run, CCA was recalculated by using only those parameters, which turned out to be significant in the forward selection. Only variables with inflation factors <10 were considered for further interpretation. Through permutation tests (function: anova.cca), the significance of the model of the first axis and of the selected environmental parameters were tested. Graphs were created in R (Murrell 2006), Windows Excel (version 14.4.9, 2011) and Sigmaplot (version 11.0) and edited in Adobe Illustrator CS6 (version 16.0.0).

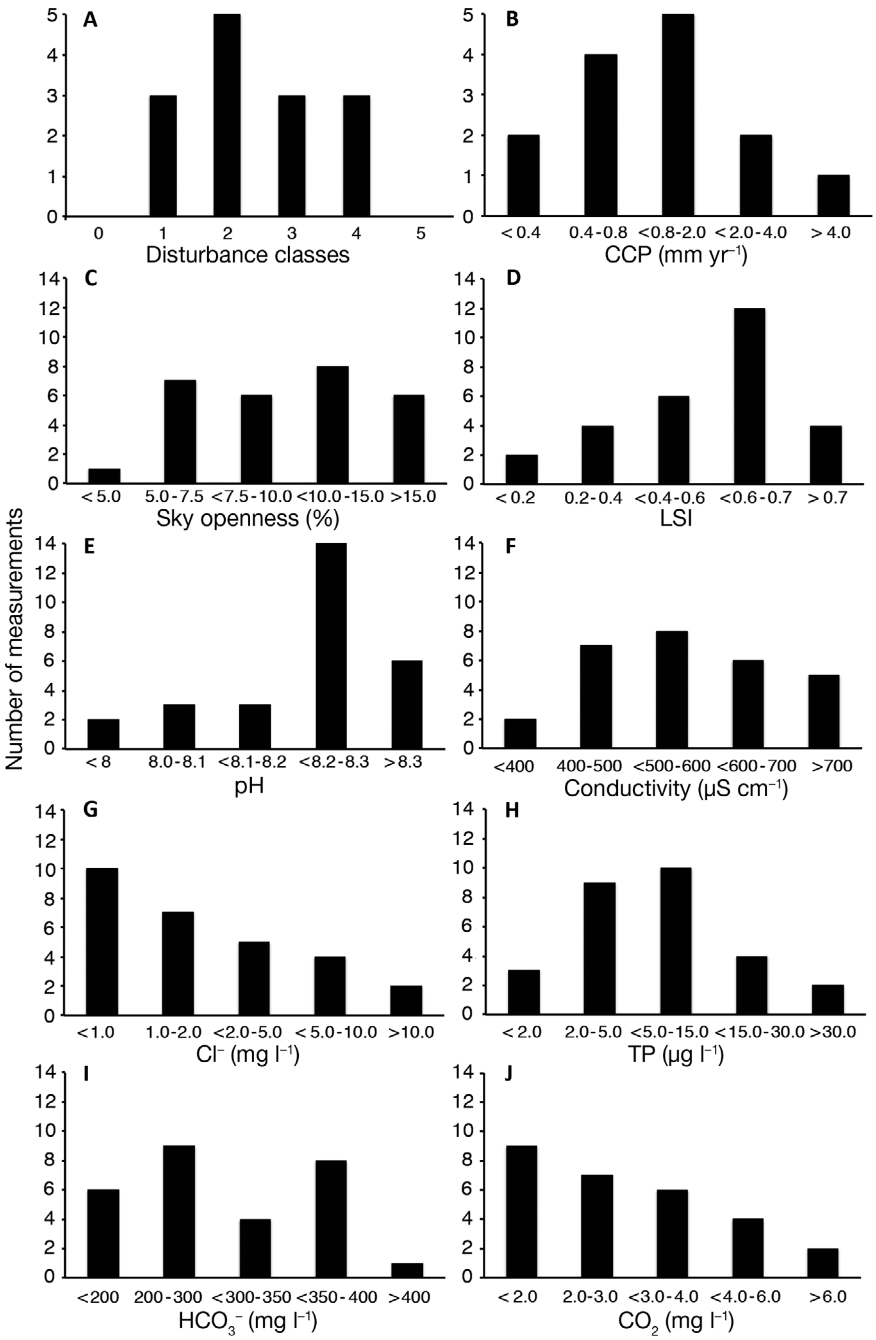

Fig. 2. Frequency histograms of environmental charactestics of 14 spring-associated limestone habitats in Austria: (A) disturbance class (ranging from 0 = no disturbance to 5 = heavily disturbed); (B) calcium carbonate precipitation rate (CCP); (C) sky openness; (D) Langelier Saturation Index (LSI); (E) pH; (F) conductivity; (G) chloride (Cl^-); (H) total phosphorus (TP); (I), bicarbonate (HCO_3^-); (J) carbon dioxide (CO_2). Panels (A) and (B) are based on 1 measurement per site and therefore contain a total of 14 measurements; the remaining panels are based on 2 measurements per site and contain a total 28 measurements

RESULTS

The environment

All of the sites were at least slightly disturbed; most of them showed moderate impacts (Fig. 2A). Sky openness ranged between 5% and 35% (Fig. 2C); CCP ranged between 0.8 and 2.0 mm yr^{-1} (Fig. 2B), which was in agreement with LSI values greater than 0, indicating carbonate precipitation (Fig. 2D). pH was within a very narrow range of 8.2 to 8.4; specific conductivity mainly showed values of 500 to 600 µS cm^{-1} with Cl^- concentrations between 0.5 and 5.0 mg l^{-1} and—with a few exceptions — low TP amounts between 5 and 15 µg l^{-1} (Fig. 2E–H). HCO_3^- concentrations at most sites were between 200 and 400 mg l^{-1} (Fig. 2I), free CO_2 was detectable, but generally below 2 mg l^{-1} (Fig. 2J). Ion composition revealed Ca^{2+} and Mg^{2+} as dominant cations; K^+ and Na^+ were of minor importance (Fig. 3A). HCO_3^- was the dominant anion; at some sites, SO_4^{3-} was also detected in higher quantities (Fig. 3B).

Species patterns

Among the main microhabitats, only travertine showed visible algal cover; gravel and sand contained only detritus including very few dead algae. In total, 93 algal taxa were observed (see Table S1 in the Supplement at www.int-res.com/articles/suppl/a080p061_supp.xls). The most abundant group was the Bacillariophyceae, comprising 76 taxa, which together represented 75 to 94% of the relative frequency of occurrence. Samples collected in May–July had higher biodiversity compared to the autumn samples (Fig. 4). Among the studied sites, Poe had the highest number of taxa (32 taxa) followed by Lut and Lud. *Achnanthidium minutissimum* complex, *Navicula* cf. *tripunctata* (Fig. 5M), *Denticula tenuis* (Fig. 5I), *Diploneis* cf. *krammeri*, *Cocconeis placentula* var. *lineata* and *Encyonopsis microcephala* (Fig. 5D) were found at 10 to 13 locations during the first sampling. *Oocardium stratum* was collected from 6 sites between May and July and 8 sites in September; 3 of the sites (Alm, Poe, Dan) represent new discoveries for this desmid.

Linking species patterns to the environment

Based on similarities of the algal communities, 7 groups were classified ($R = 0.9305$, $p < 0.001$) with merged data from both sampling dates (Fig. 6). Moreover, a geographical pattern was obvious: sites located in western Austria were clearly separated from other locations. West Austria sites (Lin, Hoc, Zoo and Lap) clustered in 2 groups, whereas all locations of Upper Austria (Edl, Mar, Dan) were placed in a single group (which also included Tes). Both Lunz sites (Lut, Lud) were merged in a single group, and Alm and Poe were also grouped together. Woe was treated as a group on its own, as was Pre. Table 2 summarizes environmental ranges of groups (for details of individual sites refer to Table S2 in the Supplement).

The indicator species analysis identified 22 indicator species (Table 3), some of them highly indicative

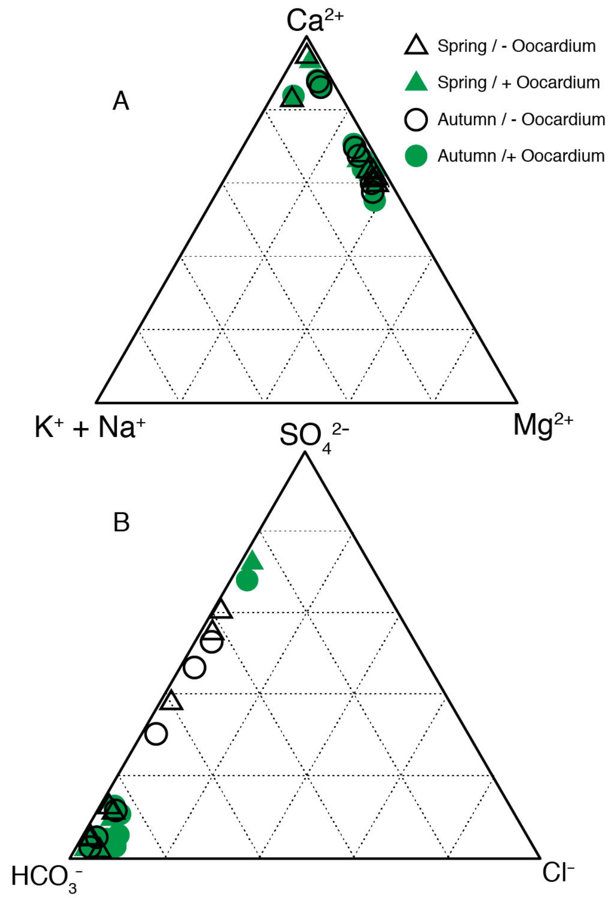

Fig. 3. Ion composition of 14 spring-associated limestone habitats in Austria, represented as ternary charts of the main (A) cations and (B) anions on a relative scale based on mg l^{-1}. Triangles and circles show the results of spring samples and autumn samples respectively. Presence and absence of *Oocardium stratum* are indicated by filled green and open symbols, respectively

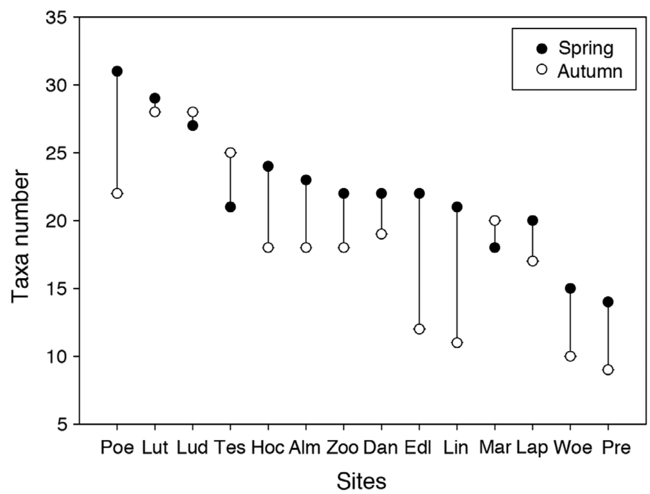

Fig. 4. Taxa richness of 14 spring-associated limestone habitats in Austria, differentiated by sampling date (spring or autumn). A total of 93 taxa were found. See Table 1 for site name abbreviations and Fig. 1 for site locations

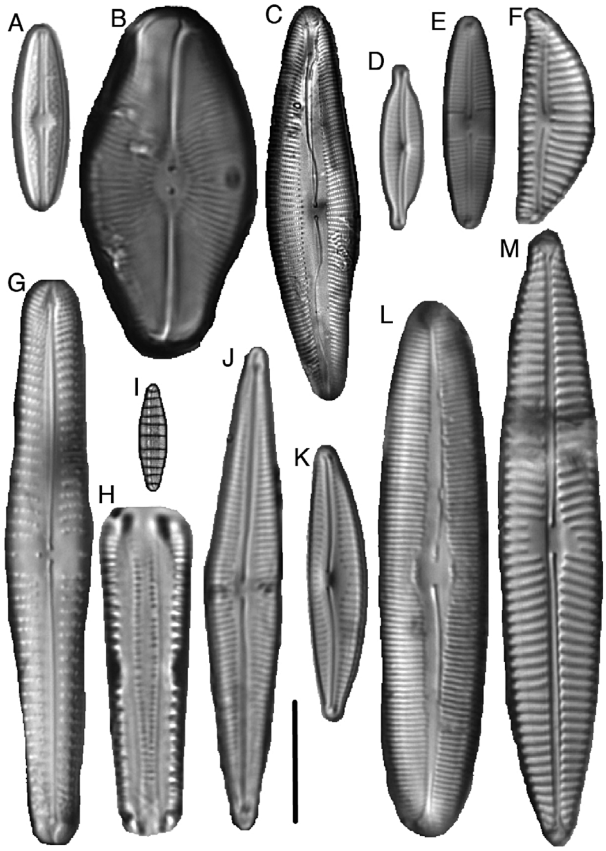

Fig. 5. Some characteristic diatoms found in spring-associated limestone habitats in Austria: (A) *Brachysira calcicola* ssp. *pfisteri*; (B) *Eucocconeis flexella*; (C) *Cymbopleura austriaca*; (D) *Encyonopsis microcephala*; (E) *Caloneis bacillum*; (F) *Encyonema minutum*; (G) *Gomphonema lateripunctatum*, valve view; (H) *Gomphonema lateripunctatum*, girdle view; (I) *Denticula tenuis*; (J) *Encyonopsis cesatii*; (K) *Delicata delicatula*; (L) *Caloneis alpestris*; (M) *Navicula* cf. *tripunctata*. Scalebar = 10 µm

for groups (Fig. 7B) such as *Nitzschia monachorum*, which occurred exclusively in Group 1 and only with high abundances (indicator value 100%). *Fallacia* cf. *lenzii* was only observed in Group 7, but with a patchy distribution resulting in a lower indicator value of 63%. *Brachysira calcicola* ssp. *pfisteri* (Fig. 5A) occurred in Groups 4 and 5 and had a higher indicator value than *Epithemia adnata*, which occurred exclusively in Group 4. *N.* cf. *tripunctata* was indicative for Group 7, but occurred also in all other groups, although in low abundance (indicator value 29.1%).

The CCA model included 25 environmental variables, with 9 contributing significantly to the species pattern ($p < 0.05$): i.e. disturbance ($F = 3.55$), CCP ($F = 2.61$), HCO_3^- ($F = 2.02$), sky openness ($F = 2.11$), pH ($F = 2.35$), LSI ($F = 1.94$), TP ($F = 1.78$), conductivity

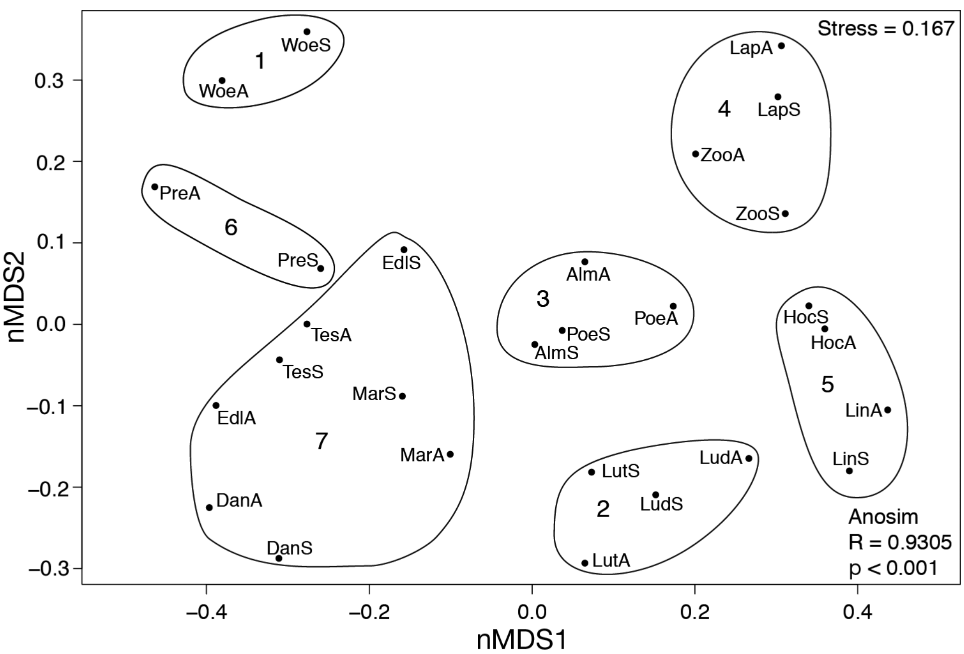

Fig. 6. nMDS of 14 spring-associated limestone (SAL) habitats in Austria based on the species occurrences. Ellipses delineate 7 groups identified by cluster analysis. An analysis of similarities (anosim) was used to test for significant differences among identified groups. For site abbreviations see Table 1, for site locations see Fig. 1, and for a synoptic list of groups of sites identified see Table 2. The letters 'S' and 'A' following the site name abbreviations indicate spring and autumn samples, respectively

Table 2. Synoptic list of the 7 groups of sampled spring-associated limestone sites in Austria identified by cluster analysis (see Fig. 6), showing, for each group, ranges of selected environmental variables, dominant (D) and eudominant (ED) algae species, and moss communities (Müller, 1991). CCP: calcium carbonate precipitation rate; Sky: sky openess; TP: total phosphorus; LSI: Langelier Saturation Index. For site abbreviations see Table 1; for locations see Fig. 1

Group	Site(s)	Parameter (min.–max.) Disturb. class	CCP (mm yr^{-1})	Sky (%)	pH	Conduct. (µS cm^{-1})	Cl^- (mg l^{-1})	TP (µg l^{-1})	HCO_3^- (mg l^{-1})	LSI	Algae	Moss
1	Woe	2–2	0.6–0.6	3.48–8.69	8.21–8.27	710–715	9.772–9.987	10–48.16	297.1–465.5	0.58–0.87	*Achnanthidium minutissimum* (D), *Phormidium nigrum* (D)	*Neckera complanata, Hypnum cupressiforme, Eurhynchium hians, Cratoneuron filicinum*
2	Lut, Lud	2–3	0.03–2.43	6.77–24.1	7.634–8.55	410–561	0.474–1.295	4–29.19	261.7–375.2	0.26–1	*Denticula tenuis* (D), *Oocardium stratum* (D), *Phormidium nigrum* (D), *Zygnema sp.* (D)	*Cratoneuron filicinum, Fissidens dubius, Calliergonella cuspidata Palustriella commutata,*
3	Alm, Poe	2–3	0.48–1.24	6.7–41.84	8.08–8.26	450–620	1.66–2.764	1–25.05	256.8–411.8	0.45–0.74		*Platyhypnidium riparioides, Pellia endiviifolia, Palustriella commutata*
4	Zoo, Lap	1–1	0.41–1.48	6.69–10.17	8.21–8.31	511–1009	0.738–6.501	1.494–10.23	147.0–204.4	0.32–0.57	*Achnanthidium minutissimum* (D), *Oocardium stratum* (D), *Ulnaria ulna* (D)	*Palustriella commutata, Eucladium verticillatum, Hymenostylium recurvirostre*
5	Hoc, Lin	1–2	2.87–4.15	8.8–17.51	8–8.323	437–630	0.772–16.81	1–10.23	275.1–393.5	0.58–0.72	*Oocardium stratum* (D)	*Plagiomnium rostratum, Ctenidium molluscum, Philonotis calcarea, Palustriella commutata*
6	Pre	2–2	0.12–0.12	4.24–5.19	8.202–8.31	264–386	0.622–0.845	2.644–25	160.4–172.6	0.12–0.23	*Diatoma mesodon* (D), *Phormidium nigrum* (D)	*Palustriella commutata*
7	Mar, Dan, Tes, Edl	3–4	0.46–1.58	4.14–33.01	8.123–8.37	433–715	1.196–2.886	0.46–179.1	180–408.2	0.46–0.78	*Phormidium nigrum* (ED), *Phormidium retzii* (D), *Vaucheria geminata* (D)	*Palustriella commutata, Calliergonella cuspidata, Brachythecium rivulare, Leucodon sciuroides*

Table 3. Indicator algal species identified at 7 groups of spring-associated limestone habitats in Austria (see Table 2), showing indicator values and abbreviation codes used in Fig. 7. Only species with a p-value < 0.05 are shown

Species	Abbreviation	Group	Indicator value (%)
Nitzschia monachorum	Nitz_mona	1	100.0
Gomphonema micropus	Gomp_micr	1	71.4
Gomphonema angustatum	Gomp_angu	1	66.7
Eunotia arcus	Euno_arcu	2	75.0
Amphipleura pellucida	Amph_pell	2	70.0
Cymbella helvetica	Cymb_helv	2	66.7
Cyclotella cretica var. *cyclopuncta*	Cycl_cycl	2	62.5
Meridion circulare	Meri_circ	2	51.9
Denticula tenuis	Dent_tenu	2	43.6
Cymbella excisiformis	Cymb_exci	2	40.0
Cymbopleura subaequalis	Cymb_suba	3	100.0
Diploneis separanda	Dipl_sepa	3	44.4
Diatoma tenuis	Diat_tenu	4	92.3
Brachysira calcicola ssp. *pfisteri*	Brac_calc	4	81.8
Epithemia adnata	Epit_adna	4	75.0
Encyonopsis microcephala	Ency_micr	4	36.4
Spirogyra sp.	Spir_sp.	5	64.3
Diatoma mesodon	Diat_meso	6	60.4
Caloneis lancettula	Calo_lanc	6	52.2
Fallacia cf. *lenzii*	Falla_lenz	7	62.5
Encyonema minutum	Ency_minu	7	45.0
Navicula cf. *tripunctata*	Navi_trip	7	29.1

($F = 1.91$) and Cl^- ($F = 1.89$). The first 4 axes explained 34% of the total variance in the data set ($p = 0.001$) (Table 4). Fig. 7A displays the first 2 canonical axes with all species (93 species) considering their affiliation to the 7 groups and the significant environmental parameters. Taxa representing Group 1 were associated with greater HCO_3^- concentrations but low conductivity, taxa in Group 7 occurred in springs with greater levels of disturbance and increased TP concentrations. Group 4 taxa experienced greater conductivity, while Group 5 had elevated Cl^-, CCP and low TP concentrations. Group 2 is distributed around the axes origin indicating no special preference towards selected parameters.

O. stratum occurred in springs with greater calcium carbonate precipitation rate, but low TP concentrations and disturbance (Fig. 7A). *O. stratum* did not show a distinct pattern concerning cation ratios, but most populations of *O. stratum* colonized springs with elevated HCO_3^- concentrations and one location with increased SO_4^{2-} concentration (Fig. 3B). We identified 16 bryophyte species occurring at the 14 sites with *Palustriella commutata* being the most common one occurring in 6 groups.

DISCUSSION

The environment

The main substrate was travertine. If sand was present, it had obviously entered the system only a short time previously; the longer it was exposed in the water, the more it was agglutinated with carbonate. Gravel was also transformed into the travertine microhabitat within a few weeks.

Almost all headwater streams of this study are located on carbonate bedrock (exceptions: Woe and Lin are tertiary/quaternary sediments; Alm is crystalline rock), which was mirrored in parameters such as alkalinity and pH. The carbonate systems were especially well buffered, which was also shown by the narrow pH ranges obtained in the current study comparable to other studies in carbonate systems (Cantonati & Ortler 1998, Arp et al. 2001, Cantonati et al. 2006).

Springs and headwaters are often oligotrophic (Cantonati & Ortler 1998, Cantonati et al. 2006, Kociolek & Stoermer 2009), which was also confirmed in our study. We found slightly increased TP values and one very high concentration of around 105 µg l^{-1} at Dan, which was attributed to run-off from surrounding agricultural land. Elevated SO_4^{2-} concentrations were measured for Zoo, Mar, Lap and Edl springs and were most probably of geogenic origin (Hobiger et al. 2007). Cl^- concentrations were low and fitted well into the classification of Wilhelm (1956), who investigated German headwaters: springs of the Alps had Cl^- concentrations of around 4 mg l^{-1}, while corresponding values for groundwater of diluvial and alluvial gravel and springs of tertiary bedrock were 8 mg l^{-1} and >14 mg l^{-1}, respectively. We found significantly increased Cl^--amounts during the first sampling survey because of snowmelt (paired Wilcoxon-test, $p = 0.03$, $n = 14$), which is in accordance with observations of Wilhelm (1956), who detected seasonal fluctuations with elevated values during spring.

Dissolved organic carbon in springs is usually <1 mg l^{-1}, increased concentrations are mainly attributed to snow melt and heavy rains (Cantonati et al. 2006). This pattern was also observed in our study: before September sampling started, heavy rains took place, which resulted in elevated values at sites Woe, Poe, Lin and Pre (May to July: 0.83 to 3.61 mg l^{-1}; September: 1.14 to 4.17 mg l^{-1}).

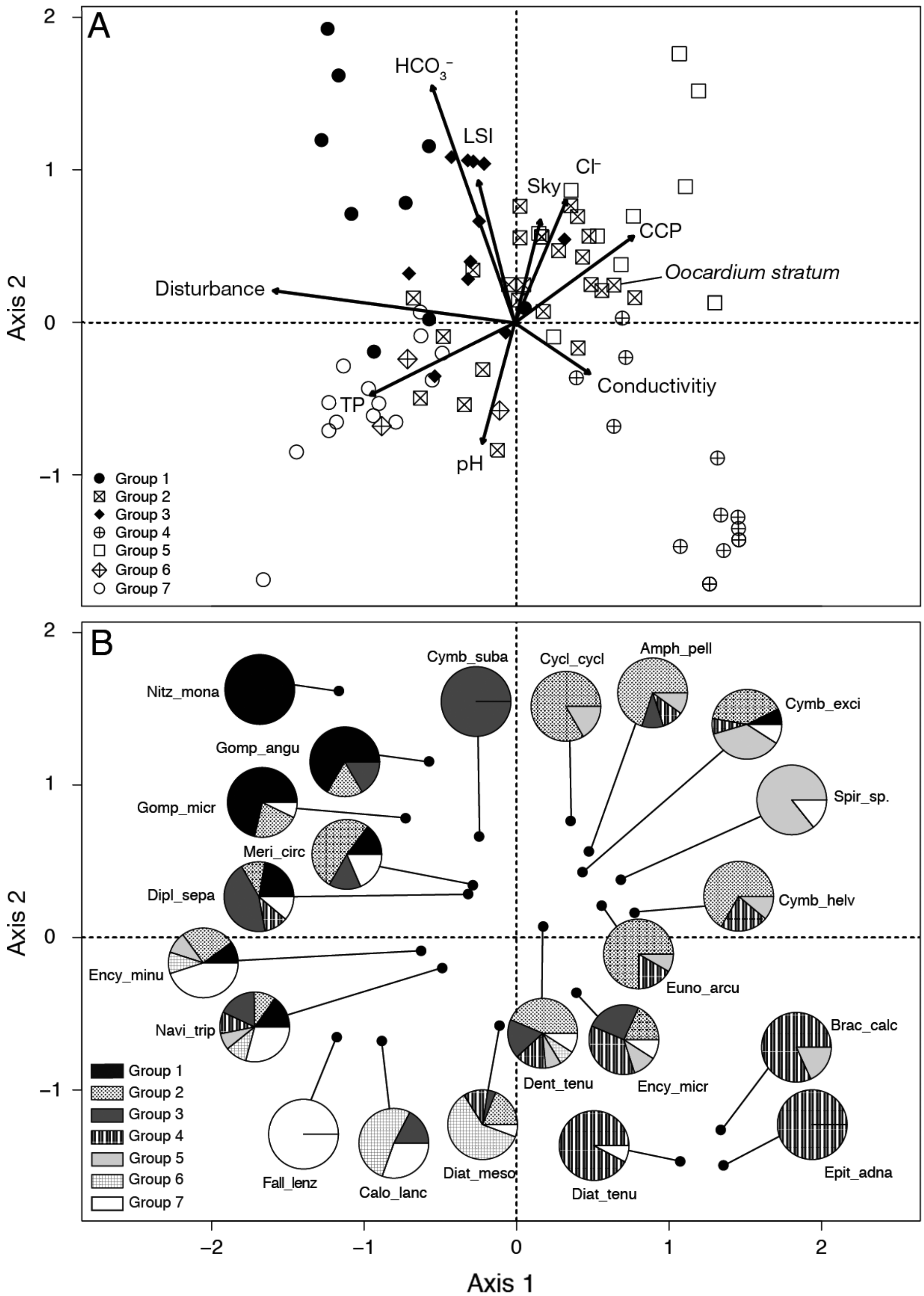

Fig. 7. (A) Constrained correspondence analysis (CCA) plot of Axes 1 and 2 identified by CCA, showing the species distribution patterns with their group membership from the cluster analysis and the significant environmental parameters with their direction. CCP: calcium carbonate precipitation rate; sky: sky openness; Cl^-: chloride; TP: total phosphorus; HCO_3^-: bicarbonate; LSI: Langelier Saturation Index. The 2 Axes explain 20.6% of the total variance. (B) The dots and the pie charts can be seen as pins. The dots indicate the locations of the indicator species in the ordination and the pie charts show the relative abundances of the species in the 7 groups of spring-associated limestone habitats. For abbreviations of species names see Table 3

Table 4. Summary statistics of the constrained correspondence analysis used to analyse associations between environmental variables and algal taxa present in spring-associated limestone habitats in Austria (see also Fig. 7)

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.401	0.286	0.255	0.202
Species–environment correlations	0.97	0.97	0.96	0.93
Cumulative percentage variance of species–environment relation	12.0	20.6	28.2	34.2
Sum of all eigenvalues	3.346			
Test of significance of the first canonical axis: p-value	0.001			
Test of significance of all canonical axes: p-value	0.001			

Species patterns

Encyponopsis microcephala (Fig. 5D) was found most frequently (19 times out of 28), followed by *Delicata delicatula* (14) (Fig. 5K), *Cymbopleura austriaca* (12) (Fig. 5C), *Caloneis bacillum* (Fig. 5E) and *Gomphonema angustum* (6 times each), *Caloneis alpestris* and *Fallacia lange-bertalotti* (5 times each) (Fig. 5L), *Cymbopleura subaequalis* and *Diploneis minuta* (4 times each). *Encyonopsis cesatti* (Fig. 5J) was found only once. The most abundant species were *Achnanthidium minutissimum*, *Navicula* cf. *tripuncata* and *Denticula tenuis* (Table S1). *A. minutissimum* is a very common epilithic species complex of running waters. Potapova & Hamilton (2007) described 6 morphological groups of the *A. minutissimum* complex from North American running waters, each with clear ecological preferences, but it was not possible to clearly delineate the groups from distinct morphological features. *N.* cf. *tripunctata* — also listed in Pentecost (1991) — indicates slight eutrophication (Krammer & Lange-Bertalot 2007b). *D. tenuis* is a character species of SAL with a worldwide distribution (Reichardt 1994, Pentecost & Zhang 2000). This taxon also shows cryptic diversity (Hamsher et al. 2014). We found *D. tenuis* at 13 sites in May–July, but only at 5 locations in September (Table S1), which points towards the need for repeated sampling throughout the year.

Cantonati et al. (2012a) investigated different spring types, one of them being SAL habitats. The authors identified 13 diatom species typical of SAL habitats; 9 were also found in the current study (Table 5). Reichardt (1994, 1995) investigated SAL habitats in Germany and identified 15 diatom taxa typical for elevated Ca^{2+}, some of them in only low abundances. From these 15 species, 9 were also identified in this study (Table 5). For cyanobacteria and algae other than diatoms, Cantonati et al. (2012b) specified 8 SAL species. From these, we only found *Oocardium stratum.* We additionally identified 17 'non-diatom' taxa in high abundances, but these are also found in other habitats than SAL. At some sites, only a single cyanobacterial taxon occurred in very high abundance completely covering the streambed (e.g. *Phormidium nigrum*).

Linking species patterns to the environment

Sanders et al. (2011) and Pentecost (2005) described taxa that are highly involved in carbonate precipitation (*Vaucheria*, *Navicula*, *Meridion*, *Gomphonema*, *Scytonema*, *Oocardium*), but all these taxa except *Oocardium* are also present in non-travertine depositing headwaters. *Oocardium* is the only genus which is prevalent exclusively in SAL headwaters (Pfiester 1976, Pentecost 2005). In addition to the sites which were already known previously, we discovered this desmid at 3 more locations, which are new reports for Austria. Two of them were recognized in September, which confirms a growth maximum of *Oocardium* in summer/autumn (Linhart & Schagerl 2015). Rott et al. (2012) investigated calcification types of *O. stratum* and the conditions of the microhabitat. The authors discovered that out of 5 different SAL sites, Lin had the highest calcification rate and the highest amount of free CO_2, which is in accordance to our observations (Table S2). Rott et al. (2012) measured values twice as high for Ca^{2+} concentration and higher Mg^{2+} concentrations at Hoc and Zoo compared to this study, which could be due to different sampling spots: Rott et al. (2012) considered the spring mouth and the downstream limit of the calcification, whereas in this study only the main *Oocardium* section was measured. This difference suggests that ion and nutrient gradients occur along the length of SAL sites from headwaters downstream. Sanders & Rott (2009) assumed that increased amounts of Mg^{2+}, Ca^{2+}, HCO_3^- and SO_4^{2-}

Table 5. Summary of typical spring-associated limestone (SAL) communities in Austria compiled from different studies. x (·): Species (not) observed in study

Species	Cantonati et al. (2012a)	Cantonati et al. (2012b)	Reichardt (1994)	Reichardt (1995)	Present study
Achnanthes trinodis (Smith) Grunow	x	·	·	·	x
Brachysira calcicola ssp. *pfisteri* Lange-Bertalot et Werum	x	·	·	·	x
Caloneis alpestris (Grunow)	·	·	x	·	x
Caloneis bacillum (Grunow) Cleve	·	·	x	·	x
Cocconeis pseudothumensis Reichardt	·	·	x	·	·
Cymbella falaisensis (Grunow) Krammer & Lange-Bertalot	·	·	x	x	·
Cymbopleura austriaca (Grunow) Krammer	x	·	x	x	x
Cymbopleura subaequalis (Grunow) Krammer	·	·	x	·	x
Delicata delicatula (Kützing) Krammer	x	·	·	x	x
Delicata minuta K.Krammer	x	·	·	·	·
Denticula kuetzingii Grunow	x	·	·	·	·
Denticula tenuis Kützing	x	·	·	·	x
Dichothrix gypsophila Bornet & Flahault	·	x	·	·	·
Diploneis minuta J.B.Petersen	·	·	x	x	x
Encyonopsis cesatii (Rabenhorst) Krammer	x	·	·	x	x
Encyonopsis krammeri Reichardt	x	·	·	·	·
Encyonopsis microcephala (Grunow) Krammer	x	·	·	x	x
Encyonopsis minuta Krammer et E. Reichardt	x	·	·	·	x
Eolimna muraloides (Hustedt) Lange-Bertalot & Kulikovskiy	·	·	x	·	·
Fallacia lange-bertalotii (E.Reichardt) E.Reichardt	·	·	x	x	·
Fallacia sublucidula (Hustedt) Mann	·	·	x	·	·
Fragilaria distans (Grunow) Bukhtiyarova	x	·	·	·	·
Gloeocapsopsis cf. *pleurocapsoides* (Novácek) Komárek & Anagnostidis ex Komárek	·	x	·	·	·
Gloeothece confluens Nägeli	·	x	·	·	·
Gomphonema angustum C.Agardh	·	·	x	·	x
Gomphonema lateripunctatum E. Reichardt et Lange-Bertalot	x	·	·	·	x
Navicula striolata (Grunow) Lange-Bertalot	·	·	x	·	·
Oocardium stratum Nägeli	·	x	·	·	x
Phormidium incrustatum Gomont ex Gomont	·	x	·	·	·
Schizothrix tinctoria Gomont ex Gomont	·	x	·	·	·
Scytonema myochrous C.Agardh ex Bornet & Flahault	·	x	·	·	·
Tapinothrix crustacea (Woronichin) Bohunická & Johansen	·	x	·	·	·
Total	13	8	12	7	15

inhibit growth of *O. stratum*, but we still observed *O. stratum* at elevated SO_4^{2-} concentrations (Fig. 3B). For *O. stratum*, Linhart & Schagerl (2015) identified water temperature and HCO_3^- concentration being key variables influencing growth at a single site. We considered more sites and found *O. stratum* generally preferring sites of low TP concentration combined with increased calcium carbonate precipitation rate.

Our results did not reveal temporal differences in taxa composition, although only 2 sampling dates were used in the present study (Fig. 5). This is in accordance with findings of Cantonati (1999). Taxa number was higher for most sites in May to July (Fig. 3), which could be an effect of ecological succession, but this hypothesis needs to be further investigated in future studies.

Larned (2010) described 5 general classes of environmental variations affecting community composition and growth of periphyton: disturbances, stressors, resources, hydraulic conditions, and biotic interactions. For spring periphyton, these parameters can be narrowed down to nutrients such as TP, geogenic factors (pH, conductivity, HCO_3^-, SO_4^{2-}) and light supply (Cantonati 2008, Gesierich & Kofler 2010, Cantonati et al. 2012b). Additionally, Cantonati et al. (2012b) found increased pH and conductivity in SAL habitats compared to other spring types. A study of diatoms in Pyrenean springs performed by Sabater & Roca (1990) revealed mineral content of the water as a key parameter for community structure. This result is in accordance to Soininen (2007), who found ion concentration and nutrient availability as most important factors for freshwater diatom distribution.

Although we focused only on the single spring type SAL sites, light supply, anions and conductivity, carbonate precipitation and pH turned out to be significant parameters for species patterns. Additionally, anthropogenic disturbance is highly influential on the community structure.

Springs are treated as ecological units (Gomi et al. 2002, Lowe & Likens 2005). Springs and adjacent headwaters can be seen as islands in the landscape with low interaction between each other and with stable conditions (Table S2), which is also reflected in the spatial species pattern we found and the absence of a temporal one (Fig. 6). The stable environment of permanent springs and adjacent headwaters and their manifold microhabitats available shape the benthic algal communities. Hampering the stability causes shifts in community composition, which can lead to loss of rare species and the reduction of ecosystem services (Covich et al. 2004). The importance of the variable 'anthropogenic disturbance' clearly revealed that it is not only the conservation of these ecosystems themselves, but also the protection of the surrounding land, which is essential for keeping these sensitive habitats intact.

Acknowledgements. This study was partly funded by the University of Vienna and the Department of Limnology and Bio-Oceanography Vienna. We thank Hubert Kraill for ion and nutrient analyses. We are grateful to Robert Krisai for the bryophyte identification. Special thanks to Rudolf Pavuza and Petra Cech from the Natural History Museum of Vienna for providing valuable information and experience concerning meteogene travertine sites in Austria.

LITERATURE CITED

Adams J (1908) A synopsis of Irish algae, freshwater and marine. Proc R Ir Acad B 27:11–60

Arp G, Wedemeyer N, Reitner J (2001) Fluvial tufa formation in a hard-water creek (Deinschwanger Bach, Franconian Alb, Germany). Facies 44:1–22

Borcard D, Gillet F, Legendre P (2011) Numerical ecology with R. Springer, New York, NY

Cantonati M (1999) Distribution and seasonality of the phytobenthos along 2 mountain spring streams in catchments of contrasting lithology. Boll Mus Civ Stor Nat Venezia 49:357–362

Cantonati M (2008) Cyanoprokaryotes and algae other than diatoms in springs and streams of the Dolomiti Bellunesi National Park (Northern Italy). Algol Stud 126:113–136

Cantonati M, Ortler K (1998) Using spring biota of pristine mountain areas for long-term monitoring. In: Kovar K et al. (eds) Proc HeadWater '98 Conf, Merano, April 1998. Publication 248, International Association of Hydrological Sciences (IAHS), Wallingford, p 379–385

Cantonati M, Gerecke R, Bertuzzi E (2006) Springs of the Alps — sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. Hydrobiologia 562:59–96

Cantonati M, Angeli N, Bertuzzi E, Spitale D, Lange-Bertalot H (2012a) Diatoms in springs of the Alps: spring types, environmental determinants, and substratum. Freshw Sci 31:499–524

Cantonati M, Rott E, Spitale D, Angeli N, Komárek J (2012b) Are benthic algae related to spring types? Freshw Sci 31: 481–498

Covich AP, Austen MC, Barlocher F, Chauvet E, and others (2004) The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. Bioscience 54: 767–775

DIN EN 1484 Wasseranalytik — Anleitungen zur Bestimmung des gesamten organischen Kohlenstoffs (TOC) und des gelösten organischen Kohlenstoffs (DOC)

DIN EN 26777:1993-04 Bestimmung von Nitrit, Spektrometrisches Verfahren (ISO 6777:1984)

Douglas B (1958) The ecology of the attached diatoms and other algae in a small stony stream. J Ecol 46:295–322

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymetrical approach. Ecol Monogr 67:345–366

Eloranta P, Kwandrans J, Kusel-Fetzmann E (2011) Rhodophyta and Phaeophyceae. Süßwasserflora von Mitteleuropa, Bd 7. Springer Spektrum, Wiesbaden

Esser K (1982) Cryptogams: cyanobacteria, algae, fungi, lichens: textbook and practical guide. Cambridge University Press, Cambridge

European Union (1992) Council Directive 92/ 43/ EEC of 21 May 1992 on the conservation of natural habitat and of wild fauna and flora. Off J Eur Commun L 206:7–50

European Union (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off J Eur Commun L 327:1–73

Frahm JP, Frey W (1992) Moosflora. Ulmer, Stuttgart

Frazer GW, Canham CD, Lertzman KP (1999) Gap Light Analyzer (GLA), Version 2.0: imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, NY

Gesierich D, Kofler W (2010) Epilithic diatoms from rheocrene springs in the Eastern Alps (Vorarlberg, Austria). Diatom Res 25:43–66

Golubić S, Violante C, Plenković-Moraj A, Grgasović T (2008) Travertines and calcareous tufa deposits: an insight into diagenesis. Geol Croat 61:363–378

Gomi T, Sidle RC, Richardson JS (2002) Understanding processes and downstream linkages of headwater system. Bioscience 52:905–916

Hamsher SE, Graeff CL, Stepanek JG, Kociolek JP (2014) Frustular morphology and polyphyly in freshwater *Denticula* (Bacillariophyceae) species, and the description of *Tetralunata* gen. nov. (Epithemiaceae, Rhopalodiales). Plant Ecol Evol 147:346–365

Hobiger G, Klein P, Haslinger E, Pirkl H (2007) Hydrochemical geogenic background values of near-surface groundwater bodies. In: Hydrologischer Atlas Österreich. Federal Ministery of Agriculture, Forestry, Environment and Water Management, Vienna

Hofmann G, Werum M, Lange-Bertalot H (2011) Diatomeen im Süßwasser — Benthos von Mitteleuropa: Bestimmungsflora Kieselalgen für die ökologische Praxis: über 700 der häufigsten Arten und ihre Ökologie. Gantner, Rugell

Hütter LA (1994) Wasser und Wasseruntersuchung: Methodik, Theorie und Praxis chemischer, chemisch-physikalischer, biologischer und bakteriologischer Untersuchungsverfahren. Otto Salle, Frankfurt am Main

Ilmonen J, Mykrä H, Virtanen R, Paasivirta L, Muotka T (2012) Responses of spring macroinvertebrate and bryophyte communities to habitat modification: community composition, species richness, and red-listed species. Freshw Sci 31:657–667

Kent M, Coker P (1992) Vegetation description and analysis; a practical approach. CRC Press, Boca Raton, FL

Kløve B, Ala-aho P, Bertrand G, Boukalova Z, and others (2011) Groundwater dependent ecosystems. Part I: hydroecological status and trends. Environ Sci Policy 14: 770–781

Kociolek JP, Stoermer EF (2009) Oligotrophy: the forgotten end of an ecological spectrum. Acta Bot Croat 68:465–472

Komárek J, Anagnostidis K (2005) Cyanoprokaryota 2: Oscillatoriales. Süßwasserflora von Mitteleuropa, Bd 19/2. Fischer, Stuttgart

Komárek J, Anagnostidis K (2013) Cyanoprokaryota 3: Heterocytous genera. Süßwasserflora von Mitteleuropa, Bd 19/3. Fischer, Stuttgart

Krammer K (1997) Die cymbelloiden Diatomeen, Teil 1. Eine Monographie der weltweit bekannten Taxa. Allgemeines und Encyonema part. Cramer, Berlin

Krammer K, Lange-Bertalot H (2004) Bacillariophyceae 4: Achnanthaceae. Süßwasserflora von Mitteleuropa, Bd 02/4. Fischer, Stuttgart

Krammer K, Lange-Bertalot H (2007a) Bacillariophyceae 2: Bacillariaceae, Epithemiaceae, Surirellaceae. Süßwasserflora von Mitteleuropa, Bd 02/2. Fischer, Stuttgart

Krammer K, Lange-Bertalot H (2007b) Bacillariophyceae 1: Naviculaceae. Süßwasserflora von Mitteleuropa, Bd 02/1. Fischer, Stuttgart

Krammer K, Lange-Bertalot H (2008) Bacillariophyceae 2: Centrales, Fragilariaceae, Eunotiaceae. Süßwasserflora von Mitteleuropa, Bd 02/02. Fischer, Stuttgart

Kresic N (2010) Types and classifications of springs. In: Kresnic N, Stevanovic Z (eds) Groundwater hydrology of springs: engineering, theory, management and sustainability. Butterworth-Heinemann, Boston, p 31–85

Larned ST (2010) A prospectus for periphyton: recent and future ecological research. J N Am Benthol Soc 29: 182–206

Linhart C, Schagerl M (2015) Seasonal succession of the travertine-forming desmid *Oocardium stratum.* J Phycol 51:1055–1065

Lowe WH, Likens GE (2005) Moving headwater streams to the head of the class. Bioscience 55:196–197

Mathews HL, Prescott GW, Obenshain SS (1965) The genesis of certain calcareous floodplain soils of Virginia. Proc Soil Sci Soc Am 29:729–732

Matoničkin I, Pavletić Z (1961) Epibiontische Verhältnisse auf den Kalktuffwasserfällen des Flusses Krka in Dalmatien. Int J Aquat Sci 18:219–224

McCune B, Grace JB, Urban DL (2002) Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR

Merz M, Zankle H (1991) The influence of the sheath on carbonate precipitation by cyanobacteria. Boll Soc Paleontol Ital 1:325–331

Murrell P (2006) R graphics. CRC Press, Boca Raton, FL

Müller HJ (1991) Ökologie. G. Fischer, Jena

OENORM EN ISO 10304-1:2012-06-01 Bestimmung von gelösten Anionen mittels Flüssigkeits-Ionenchromatographie — Teil 1: Bestimmung von Bromid, Chlorid, Fluorid, Nitrat, Nitrit, Phosphat und Sulfat (ISO 10304-1:2007 + Cor 1:2010)

OENORM EN ISO 14911:1999-11-01 Bestimmung der gelösten Kationen Li^+, Na^+, NH_4^+, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, und Ba^{2+} mittels Ionenchromatographie — Verfahren für Wasser und Abwasser (ISO 14911:1998)

OENORM EN ISO 6878:2004-09-01 Bestimmung von Phosphor — Photometrisches Verfahren mittels Ammoniummolybdat

OENORM ISO 7150-1:1987-12-01 Bestimmung von Ammonium, manuelle spektrophotometrische Methode

Pentecost A (1981) The tufa depositis of the Malham district. Field Stud 5:365–387

Pentecost A (1991) Algal and bryophyte flora of a Yorkshire (U.K.) hill stream: a comparative approach using biovolume estimations. Arch Hydrobiol 121:181–201

Pentecost A (1993) British travertines: a review. Proc Geol Assoc 104:23–39

Pentecost A (2005) Travertine. Springer, Berlin

Pentecost A, Viles H (1994) A review and reassessment of travertine classification. Geogr Phys Quat 48:305–314

Pentecost A, Zhang Z (2000) The travertine flora of Juizhaigou and Munigou, China, and its relationship with calcium carbonate deposition. Cave & Karst Science 27: 71–79

Pfiester LA (1976) *Oocardium stratum* a rare (?) desmid (Chlorophyceae). J Phycol 12:134

Pomeisl E (1953) Der Mauerbach. Wetter und Leben, Sonderheft II:103–121

Potapova M, Hamilton PB (2007) Morphological and ecological variation within the *Achnanthidium minutissimum* (Bacillariophyceae) species complex. J Phycol 43:561–575

Reichardt E (1994) Zur Diatomeenflora (Bacillariophyceae) tuffabscheidender Quellen und Bäche im Südlichen Frankenjura. Ber Bayer Bot Ges 64:119–133

Reichardt E (1995) Die Kieselalgenflora (Baciallariophyceae) des Wachsenden Steins von Usterling. Ber Bayer Bot Ges 65:87–92

Rieth A (1980) Xanthophyceae. Süßwasserflora von Mitteleuropa, Bd 4/2. Fischer, Stuttgart

Rott E, Hotzy R, Cantonati M, Sanders D (2012) Calcification types of *Oocardium stratum* Nägeli and microhabitat conditions in springs of the Alps. Freshw Sci 31: 610–624

Sabater S, Roca JR (1990) Some factors affecting distribution of diatom assemblages in Pyrenean springs. Freshw Biol 24:493–507

Sanders D, Rott E (2009) Contrasting styles of calcification by the micro-alga *Oocardium stratum* Nägeli 1849 (Zygnematophyceae) in two limestone-precipitating springs of the Alps. Aust J Earth Sci 102:34–49

Sanders D, Wertl W, Rott E (2011) Spring-associated limestones of the Eastern Alps: overview of facies, deposystems, minerals, and biota. Facies 57:395–416

Soininen J (2007) Environmental and spatial control of freshwarter diatoms — a review. Diatom Res 22:473–490

Stanford JA, Ward JV, Ellis BK (1994) Ecology of the alluvial aquifers of the flathead river, Montana. In: Gibert J, Danielopol DL, Stanford JA (eds) Groundwater ecology. Academic Press, London, p 367–390

Strahler AN (1957) Quantitative analysis of watershed geomorphology. Eos (Wash DC) 38:913–920 ter Braak CJF, Prentice IC (1988) A theory of gradient analysis. Adv Ecol Res 18:271–317

van Oye P, Hubert B (1937) Recherches sur les 'crons' du Jurassique Belge. Biologische Jaarboek Dodonaea 4: 231–236

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37:130–137

Wallner J (1933) *Oocardium stratum* Naeg., eine wichtige tuffbildende Alge Südbayerns. Z Wiss Biol 20:287–293

Wallner J (1934) Über die Verbreitungsökologie der Desmidiacee *Oocardium*. Int J Plant Biol 23:249–263

Weigand E (1998) Limnologisch-faunistiche Characterisierung von Karstquellen, Quellbächen und unterirdischen Gewässern nach Choriotopen und biozönotischen Gewässerregionen (Nationalpark o.ö. Kalkalpen, Österreich). Nationalpark o.ö. Kalkalpen, Leonstein

West GS, West W (1901) The alga-flora of Yorkshire: a complete account of the known freshwater Algae of the county, with many notes on their affinities and distribution. Taylor, Leeds

Wilhelm F (1956) Physikalisch-chemische Untersuchungen an Quellen in den bayrischen Alpen und im Alpenvorland. Münchner Geogr Hefte 10:1–97

Editorial responsibility: Tom Battin, Lausanne, Switzerland

Submitted: November 29, 2016; Accepted: May 10, 2017
Proofs received from author(s): July 30, 2017