Diatom diversity in headwaters influenced by permafrost thawing: First evidence from the Central Italian Alps

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ABSTRACT

Glacier melting and permafrost thawing are the most evident effects of the current climate change that is strongly affecting high mountain areas, including the European Alps. As the thawing rate of subsurface ice is lower than for glacier ice, it is expected that, while glaciers retreat, an increasing number of Alpine headwaters will become more influenced by permafrost degradation during the 21st century. Despite the expected change in the relative importance of glacier and permafrost in determining Alpine hydrology, studies addressing effects of permafrost thawing on chemical and, especially, biological features of adjacent surface waters are still scarce. The present study contributes to characterise the epilithic and epiphytic diatom diversity in a set of permafrost-fed headwaters in three sub-catchments differing in bedrock lithology of the Italian Central Alps (Trentino Alto-Adige) in relation to water chemistry and habitat features. In addition, it explores chemical and biological differences between permafrost-fed streams and headwaters with no direct contact to permafrost, namely glacier-fed (kryal) and precipitation-/groundwater-fed (rhithral) streams. Permafrost-fed waters showed higher electrical conductivity and enhanced ion concentrations than glacier- and precipitation-fed waters, while concentration of trace elements (*e.g.* Sr, Ni, Zn, As) were more irregularly distributed among waters of different origin, though they showed a tendency to reach higher levels in permafrost-fed waters. Diatom species richness and diversity were lower in permafrost-fed headwaters, and were principally related to water pH and trace metal concentrations. Epiphytic diatom assemblages were more diverse than epilithic ones, independently from the water origin, while differences in species composition were not sufficient to unequivocally identify a typical diatom composition for the different water types considered in this study.

Key words: Diatoms; biodiversity; Alpine headwaters; mountain permafrost; rock-glacier; water chemistry.

INTRODUCTION

Under the influence of the current global warming (IPCC, 2013), the European Alps have experienced an average temperature increase of about 2°C since the beginning of the 20th century, which is twice as much as the global average (EEA, 2009). This trend is expected to continue with an average temperature increase of 1.5°C (in comparison with 1961-1990 period) by the first half of 21st century, and up to 3.5°C during the second half (Gobiet *et al*., 2014).

The cryosphere reduction is the most evident effect of climate change in high mountain areas. Mountain glaciers are highly sensitive to the rising air temperature and their melting rate, estimated as ~2 km³ y⁻¹ in the European Alps (Haeberli *et al*., 2017), has increased since the 1980s, causing the loss of ~50% of their ice volume (Beniston *et al*., 2018). At this rate, 80-100% of the glaciers in the Alps are predicted to disappear by the end of the century (Zemp *et al.*, 2006; Slemmons *et al*., 2013), leading to alterations in hydrological regimes, water chemistry and biodiversity in glacial-fed rivers (Milner *et al.*, 2017).

Mountain permafrost (*i.e.* soil or rock that remains at or below 0°C for at least two consecutive years, Dobinski, 2011), is also an important component of the Alpine cryosphere that is threatened by climate change (Beniston *et al*., 2018), and its extent in the European Alps is estimated to exceed that of glaciers (about 6200 km² if considering an index ≥0.5, Boeckli *et al*., 2012). The most common permafrost evidence in the Alps are rock glaciers, *i.e.* landforms composed by a mixture of ice and debris and, if active, slowly creeping downslope (Schoeneich *et al*., 2011). Rock glaciers are considered as an important water reserve for mountain areas (Jones *et al*., 2018).

At present, glaciers and snowpack represent the main drivers of the Alpine hydrology, as their seasonal melting is tightly associated with surface and groundwater flow (Jansson *et al*., 2003), while permafrost thawing represents a secondary source to Alpine freshwater (Krainer *et al*., 2007; 2011). In fact, subsurface ice is considered as less climatically sensitive than glaciers (Jones *et al*., 2018) and its thawing rate is estimated to be 10-100 times slower than for glaciers due to the thermal

buffering provided by the thick debris coating (Harris and Pedersen, 1998). Nevertheless, permafrost thawing and its relative importance for the Alpine hydrology is expected to increase during the 21st century due to warmer and prolonged summers and reduced precipitation (Haeberli *et al.*, 2017).

Despite the expected change in the relative importance of the major components of the Alpine cryosphere, the understanding of the possible cascading effects of permafrost degradation on water quality and ecology of Alpine freshwater is still largely incomplete. Insights into the effects of rock glacier thawing on inorganic chemistry of Alpine headwaters were provided by some recent case studies (Krainer *et al.*, 2007; Thies *et al.*, 2007, 2013, 2018; Colombo *et al.*, 2018a; Engel *et al.*, 2018). Compared to waters fed by glacier melt or atmospheric precipitation and groundwater, headwaters emerging from active rock glaciers are commonly characterised by higher electrical conductivity (EC) and concentration of major ions, in particular Mg^{2+}, Ca^{2+} and SO_4^{2-} (Colombo *et al.*, 2018b). In addition, elevated trace metal concentrations (*e.g.* Al, Fe, Ni, Sr) are often detected in permafrost-fed headwaters, especially in metamorphic watersheds (Krainer *et al.*, 2007; Thies *et al.*, 2007, 2013; Ilyashuk *et al.*, 2014; Engel *et al.*, 2018).

In general, studies specifically addressing the impact of deglaciation on the biota of Alpine streams are scarce (Fell *et al.*, 2017; Hotaling *et al.*, 2017; Brown *et al.*, 2018), and studies on the effects of permafrost thaw on the headwater biota are still occasional. Primary producers are particularly poorly investigated, despite benthic algae (especially diatoms) play a key ecological role in Alpine freshwaters (where external organic input is usually very scarce) as energy source for consumers (Rott *et al.*, 2006). Nevertheless, first studies show that epilithic diatom assemblages of permafrost-fed running waters are mainly influenced by decrease in water pH and increase in EC and trace metals that appear to be able to induce significant changes in both species' composition and diversity (Thies *et al.*, 2013; Lösch *et al.*, 2015).

Scope of the present work is to contribute in filling the knowledge gap on diatom diversity in permafrost-fed headwaters of the Italian Central Alps (Trentino Alto-Adige) in relation to water chemistry and habitat features. The study also aims at enlarging the perspective from one to several sub-catchments and at comparing species richness and diversity of epilithic and epiphytic diatom assemblages. Considering the predicted importance of permafrost in determining hydrology, chemistry and biology of headwater in a near future characterized by disappearing glaciers, this work aims also at exploring differences between permafrost-fed streams and headwaters with no direct contact with permafrost, namely glacier-fed (kryal) and precipitation-/groundwater-fed (rhithral) streams. This aspect may be ecologically relevant as waters emerging from active rock-glaciers are considered as potential refugia for the kryal biodiversity (Hotaling *et al.*, 2017), and as possible stepping stones for the colonization of areas exposed by glacier retreat by organisms of lower altitudes.

METHODS

Study sites

A set of 16 headwaters of different origin were studied in three sub-catchments of the Italian Central Alps (Trentino Alto-Adige), *i.e.* Sulden/Solda (SU), La Mare (MR) and Amola (AM) valleys (Fig. 1 and Tab. 1).

The lithology of the studied areas consists almost exclusively of crystalline bedrocks. SU and MR valleys, which are located respectively in the northern and southern regions of the Ortles-Cevedale group and belong to the Austroalpine domain of the Central Alps, are dominated by Pre-permian metamorphic rocks, and show prevalence, respectively, of phyllades and granitic orthogneiss, and of micaschists (Montrasio *et al.*, 2012). The bedrock of the sampled area in the AM Valley, which belongs to the Southalpine domain, consists of Oligocene intrusive tonalite rocks (Callegari and Brack, 2002).

The three sub-catchments include glacial landforms of different size and with different degree of debris cover that ranges from clean glaciers, *i.e.* La Mare and Suldner/Solda glaciers (MR and SU valleys, respectively) to almost completely debris-covered glaciers, *i.e.* Amola (AM Valley) and Klein Angelus/Angelo Piccolo glaciers (SU Valley). Permafrost landforms are widespread in all the three areas, and mainly include active rock glaciers, and inactive and relict rock glaciers at lower elevations. Vegetation is absent or very sparse in the upper range of each studied area, while alpine meadows and sparse shrubs reach 2500-2600 m asl, according to the different orographic features of each sub-catchment.

The mean annual precipitation ranges from 800-950 mm y⁻¹ at the floor of the SU Valley (Adler, 2015) to ~1200 mm y⁻¹ in the AM Valley (Baroni *et al.*, 2004). Studies conducted in the past three decades indicate that these areas are affected by moderate atmospheric deposition of nitrogen (N) compounds, originating from the emission sources located in the Po Plain, one of the most densely inhabited and industrialized regions of Europe (Rogora *et al.*, 2006; Marchetto *et al.*, 2014).

Sampling and laboratory analysis

Water and diatom samples were collected in late summer 2016 (between late August and early September), under condition of maximum melt water contribution from glaciers and rock glaciers and no snowmelt, in order

to capture the highest diatom biodiversity (Rott *et al.*, 2006; Uehlinger *et al.*, 2010). Headwaters fed by permafrost and glaciers (RG and GL, respectively), were sampled at each studied sub-catchment, while rhithral waters fed by atmospheric precipitation, groundwater or of mixed origin (REF), and with no direct contact with permafrost and glaciers, could be found only in the MR and AM valleys (Fig. 1 and Tab. 1).
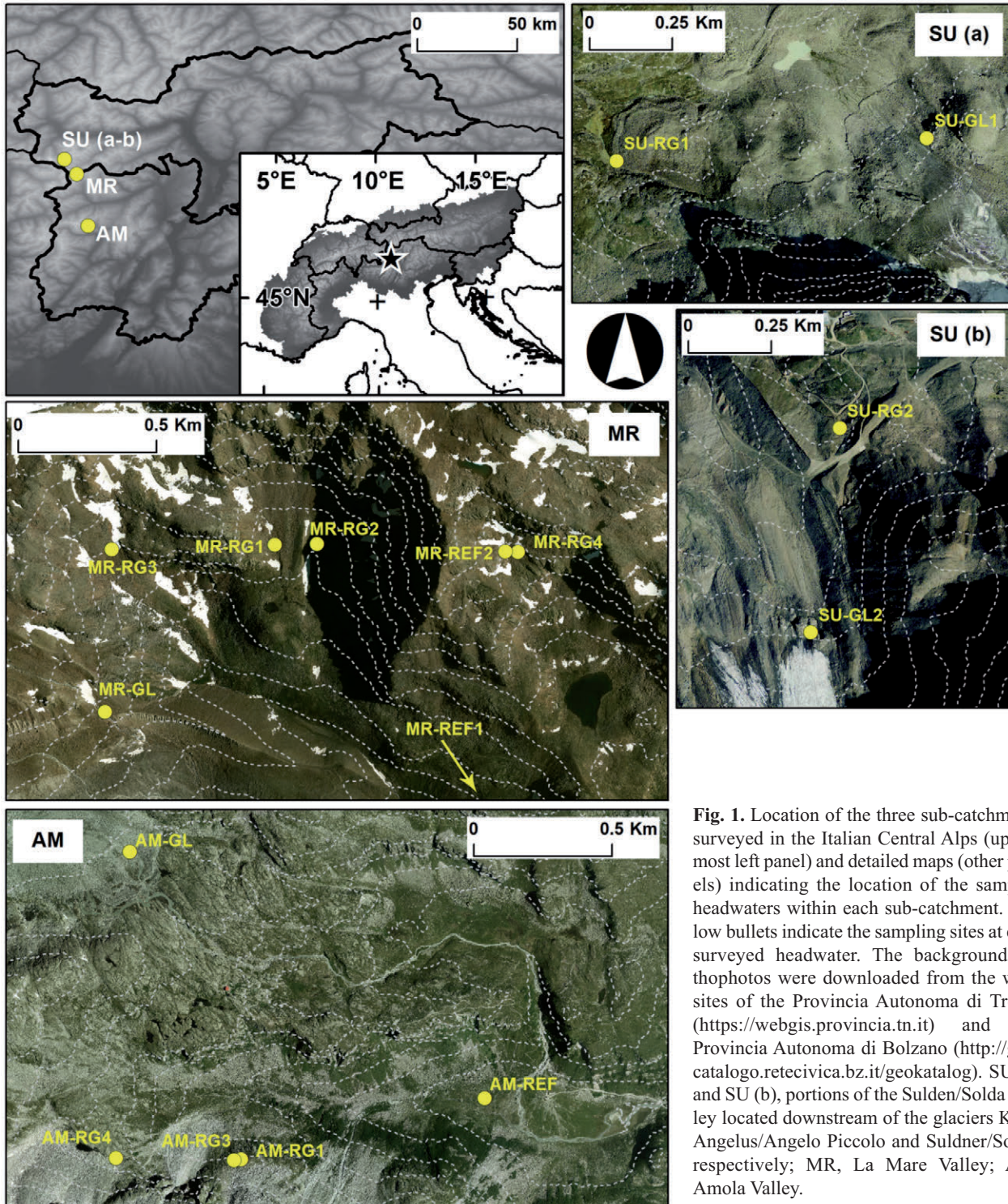

Fig. 1. Location of the three sub-catchments surveyed in the Italian Central Alps (uppermost left panel) and detailed maps (other panels) indicating the location of the sampled headwaters within each sub-catchment. Yellow bullets indicate the sampling sites at each surveyed headwater. The background orthophotos were downloaded from the websites of the Provincia Autonoma di Trento (https://webgis.provincia.tn.it) and the Provincia Autonoma di Bolzano (http://geo-catalogo.retecivica.bz.it/geokatalog). SU (a) and SU (b), portions of the Sulden/Solda Valley located downstream of the glaciers Klein Angelus/Angelo Piccolo and Suldner/Solda, respectively; MR, La Mare Valley; AM, Amola Valley.

In the SU Valley, streams fed by the Klein Angelus/Angelo Piccolo (SU-GL1) and Suldner/Solda (SU-GL2) glaciers were surveyed, together with two springs emerging from two active rock-glaciers located close to each of these two glaciers (SU-RG1 and SU-RG2, respectively, Fig. 1). As the first RG is part of the periglacial belt around the Klein Angelus/Angelo piccolo glacier, it is partially flushed by glacial melting water flowing underground (Brighenti, pers. comm). Seven different headwaters were surveyed in the MR Valley (Fig. 1). On the left side of the valley, active rock glaciers and talus slopes with probable presence of permafrost (Carturan *et al.*, 2016) originate small streams that partially flow underground due to the slope morphology. Four springs emerging from the lowest margin of rock-glaciers or talus slopes (Tab. 1) were sampled at altitudes between 2710 and 2890 m asl, while a glacial stream (MR-GL) emerging from La Mare glacier was sampled at 2898 m altitude on the right valley side (Fig. 1). Rhithral waters with no direct contact with permafrost were sampled in the upper part of the valley at 2890 m asl, and at the sub-catchment closing section at 2266 m asl (Tab. 1), upstream the confluence with the glacial stream. Discontinuous permafrost and active rock glaciers characterize the orographic right side of the AM Valley (Baroni *et al.*, 2004; Seppi *et al.*, 2012), were samples

were collected from two springs emerging from an active rock glacier (AM-RG1 and AM-RG3 around 2300 m asl, Fig. 1) and from a talus slope (AM-RG4 at 2570 m asl) characterized by a very low permafrost index according to Boeckli *et al.* (2012). The glacial stream fed by meltwater of the Amola glacier (AM-GL), located on the left of the valley, was sampled at 2427 m altitude, while the rhithral stream collecting all non-glacial waters originating from the right side of the valley (AM-REF, Fig. 1) was sampled at 2027 m asl before the confluence with the glacial stream.

Each sampled headwater has been described by location (altitude and geographic coordinates) and qualitative variables, such as water discharge and turbidity and presence of vegetation inside and outside the water channel, which were empirically estimated on site (Tab. 1). Water temperature, electrical conductivity (EC), and turbidity (the latter only for the SU and part of MR valley) were measured in the field on site with portable conductivity and turbidity meters (WTW GmBH, Weilheim, Germany). Water turbidity of the other surveyed waters and water discharge were empirically estimated by observation during the sampling; water turbidity was given a value between 0 and 5 (Tab. 1).

Water samples for chemical analyses were collected in acid washed high density polyethylene (HDPE) bottles and

Tab. 1. Location, dominant geology, habitat characteristics of the 16 headwaters surveyed in the present study, and type of diatom samples collected.

Nr	ID	Catc	Lat-X, Long-Y	Altid m a.s.l.	Geol	Veg In, Out	Turb NTU, (Est)	Temp °C	POM % DW	CO_3^{2-} % LOI	Dia
1	SU-GL1	A	625341, 5156147	2890	M, ph, go	-, -	42 (2)	3.0	10.9	0.10	E
2	SU-RG1	A	624406, 5156079	2728	M, ph, go	+, +	6 (1)	1.1	46.5	0.9	E, M
3	SU-GL2	A	622653, 5148781	2726	M, ph, go	-, -	306 (3)	0.1	7.8	6.6	E
4	SU-RG2	A	622736, 5149503	2579	M, ph, go	+, -	3 (0)	1.0	27.3	1.9	E, M
5	MR-GL	N	632904, 5118237	2898	M, ms	-, -	n.a. (4)	6.0	6.7	2.9	E
6	MR-RG1	N	627492, 5144697	2710	M, ms	-, -	n.a. (0)	2.4	25.4	3.9	E
7	MR-RG2	N	627645, 5144701	2717	M, ms	+, -	n.a. (0)	4.4	26.4	1.6	E, M
8	MR-RG3	N	626902, 5144681	2809	M, ms	-, -	n.a. (0)	0.6	21.6	5.36	E
9	MR-RG4	N	628372, 5144671	2890	M, ms	+, -	n.a. (0)	2.1	27.3	1.35	E, M
10	MR-REF1	N	628325, 5144673	2266	M, ms	+, +	n.a. (0)	10.6	52.5	12.4	E, M
11	MR-REF2	N	628325, 5144673	2898	M, ms	+, -	n.a. (0)	9.4	78.1	1.7	E, M
12	AM-GL	S	631622, 5119213	2427	I, to	-, -	n.a. (5)	2.3	12.0	1.6	E
13	AM-RG1	S	632024, 5118102	2321	I, to	+ +	n.a (0).	0.8	94.1	7.7	E, M
14	AM-RG3	S	632024, 5118102	2578	I, to	-, -	n.a. (0)	1.1	5.6	0.6	E
15	AM-RG4	S	631571, 5118106	2385	I, to	+, -	n.a. (0)	4.5	41.5	4.8	E, M
16	AM-REF	S	632904, 5118323	2027	I, to	+, +	n.a. (0)	8.1	21.7	13.9	E, M

ID, headwater code; AM, Amola Valley; MR, La Mare Valley; SU, Sulden/Solda Valley; GL, glacier-fed; RG, permafrost-fed; REF, rhithral streams fed by precipitation and/or groundwater; Catc, major river catchment to which each sampled headwater belongs; S, Sarca River; N, Noce River; A, Adige River; Lat-X and Long-Y, UTM latitude and longitude; respectively; Altid, altitude, Geol, geology of the watershed; I, intrusive; M, metamorphic; to, tonalite; ms, micaschists; ph, phyllades; go, granitic orthogneiss; Veg, vegetation present in (In) or ouside (Out) each headwater; Turb, water turbidity measured as nephelometric turbidity units (NTU) and estimated (0 to 5) by direct observation (Est); n.a., not available; Temp, water temperature; POM, particulate organic matter of the epilithic biofilm; DW, dry weight; LOI, lost on ignition; Dia, diatom samples collected from each studied headwater; E, epilithic; M, epiphytic from submerged moss.

preserved at dark and cool (4°C) until analysis. Electrical conductivity (EC) at 20°C, pH, alkalinity, major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, SO_4^{2-}, Cl^-), and nutrients (NO_3-N, NH_4-N, total nitrogen (TN), soluble (P-PO_4) and total phosphorous (TP), SiO_2) were determined according to standard methods (APHA-AWWA-WPCF, 2017) at the FEM Hydrochemistry Lab. Water samples for the analyses of trace metals were filtered in the field through 0.2 μm mesh polycarbonate filters, collected in acidified 100 mL HDPE bottles and immediately preserved adding 100 μL of HNO_3 65% (APAT, CNR-IRSA, 2003). Trace metal concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) with ultrasonic nebulizer at the water chemistry laboratory of the Water Research Institute of Verbania, Italy (CNR-IRSA), formerly Institute of Ecosystem Study (CNR-ISE). Further details on the analytical methods and the quality assurance/quality controls adopted in the laboratory can be found at http://www.idrolab.ise.cnr.it/en/.

Samples for the analysis of the epilithic diatoms assemblages were collected by brushing the surface of 10 different stones randomly collected along a ~10 m long section of each surveyed headwater, according to European Committee for Standardization (2014). An aliquot of each epilithic sample was immediately subsampled for the determination of organic and carbonate content according to Hauer and Lamberti (2011). Around 0.5 g of freeze-dried material was heated in a furnace at 550°C for 12 hours to quantify the percent content of particulate organic matter (POM), weighted and heated again at 950°C for 3 hours to determine the CO_3^{2-} content to be used as an estimate of primary production. Epiphytic diatoms were yielded, wherever possible, by collecting and washing submerged mosses located in the water flow. Epiphytic diatoms were retrieved from the majority of the permafrost-fed and rhithral waters, while water mosses were absent in all the surveyed glacier-fed streams. All diatom samples were preserved in 70% ethanol till the cleaning in hot 30% H_2O_2, and HCl 37%, and the permanent mounting in Naphrax® resin, refractive index of 1.7 (European Committee for Standardization, 2014). Oxidation of samples containing particularly resistant organic matter was obtained by adding $K_2Cr_2O_7$ grain by grain (Tolotti, 2001). According to the European Committee for Standardization (2004), at least 400 valves were identified and enumerated along transects of each permanent slide under a Leica DM2500 microscope equipped with differential interference contrast optics and a 100x oil-immersion objective (1000x magnification). On the contrary, the total area of the slides relative to the glacial-fed streams was scanned, due to the very low diatoms density. Taxon identification was based on standard literature (Krammer and Lange-Bertalot, 1986-2004;

Krammer, 2000; Lange-Bertalot, 2001; Lange-Bertalot et al., 2011, 2017), integrated by literature on recently revised taxa (Potapova and Hamilton et al., 2007; Hlúbikova et al., 2009).

Data analysis

Variability of pH, major ions responsible for EC (i.e. Ca^{2+}, Mg^{2+} and SO_4^{2-}), and more abundant nutrients (NO_3-N, TP and SiO_2) in the surveyed waters of different origin was compared with one-way ANOVA tests or ANOVA tests on ranks, after testing the normal distribution of the data through a Kolmogorov-Smirnov distance test. Pairwise multiple comparison was performed following the Holm-Sidak method (Holm, 1979).

Diatom α-diversity of epilithic and epiphytic assemblages was evaluated based on the Shannon Index (Shannon and Weaver, 1949) calculated on number of identified individuals for each taxon. The Evenness (Pielou, 1975) and Hill's index (Hill, 1973) were computed to obtain information on the heterogeneity of the assemblages. Other diatom data analyses were based on relative abundance (RA) of the diatom taxa identified. Preferences of the identified taxa for pH, total phosphorous concentrations, and moisture were evaluated according to the classification proposed by Van Dam et al. (1994) for European freshwaters.

Non-metric multidimensional scaling (NMDS; Kruskal and Wish, 1978) was performed by R 3.2.2 (R Core Team, 2015) software in order to graphically evaluate differences between diatom assemblages identified on different substrata in the studied sites. NMDS was applied to a Bray and Curtis dissimilarity matrix (Legendre and Legendre, 1998) based on non-transformed relative abundances of the 42 diatom taxa reaching RA ≥0.5% at least in two samples. These restrictions provided the analysis to be performed on an average 95% (84.7-99.7%) of the total diatom abundance of the different samples. Epilithic and epiphytic diatom assemblages were evaluated simultaneously in order to obtain a direct comparison of the two diatom communities. Samples from the four glacier-fed streams were excluded from the analyses because of the very low number of valves identified in these samples (see below). Stress values of NMDS configurations with increasing numbers of dimensions were compared in order to evaluate the stability of the analysis and to identify the minimum number of dimensions that is sufficient to explain samples variability (Legendre and Legendre, 1998). A vector fitting analysis was applied to the sample scores on the final NMDS configuration, and computed using the R function envfit included in the vegan package ver. 2.3-0 (Oksanen et al., 2015) in order to identify the maximum correlation between the sample distribution within the NMDS ordination space and habitat features

and water physical and chemical characteristics of the headwaters studied. Except for water temperature and pH, all environmental and chemical data were log transformed (y=log (x+1)) in order to reduce the variability within the data set. In addition, the relation between sample scores on the NMDS dimensions and the abundance of single diatom taxa and cumulative RA of diatoms with different preferences for pH, TP, and moisture was explored by correlation analysis. A negative Kolmogorov-Smirnov distance test indicated the Spearman rank correlation coefficient (ρ) as suitable for exploring the correlation within the data matrix.

RESULTS

Water chemistry

All permafrost-fed waters were characterised at the sampling moment by low discharge (estimated as ranging from ~ 1 L sec^{-1} for the majority of RG springs to ~5 L sec^{-1} for AM-RG4 and SU-RG1), low temperature (ranging from 0.6°C for springs to 4.4°C for waters emerging from talus slopes, *i.e.* MR-RG2 and AM-RG4), and very low water turbidity (Tab. 1). No significant differences emerged between the three studied sub-catchments. On the other hand, the glacier-fed streams showed higher discharge (estimated as between 5 and 20 L sec^{-1}), high mineral turbidity and variable water temperature in relation to the distance of the sampling site from the glacier snout (Tab. 1).

The pH values of the majority of the surveyed waters ranged from acidic to neutral, while both the headwaters sampled downstream the Suldner/Solda Glacier (*i.e.* SU-GL2 and SU-RG2) showed basic values (Supplementary Tab. 2). Although the one-way ANOVA test did not show significant differences in the median pH values of the three water types, glacier-fed and rhithral waters showed higher variability compared to permafrost-fed waters, whose 50% of pH values ranged between 6.6 and 6.9 (Fig. 2). Median alkalinity of the permafrost-fed waters (5.9±20.3 µeq L^{-1}) was slightly, but not significantly, higher and more variable than values in glacier-fed and rhithral waters (Supplementary Tab. 2).

In general, permafrost-fed waters showed high EC values and ion concentrations (median EC=155±213 µS cm^{-1}), especially in MR and SU valleys (up to 516 µS cm^{-1} EC in MR-RG4), while all headwaters surveyed in the AM Valley showed scarce mineralization, with EC values never exceeding 20 µS cm^{-1} (Supplementary Tab. 2). The ions Ca^{2+}, Mg^{2+} and SO$_4^{2-}$ were the most important contributors to the enhanced EC values recorded in the permafrost-fed waters, where they showed quite variable concentrations (Fig. 2). Due to this high variability, the ANOVA test on ranks (for non-

normally distributed data) indicated that the median concentrations of these ions in permafrost-fed waters (*i.e.* 71.0, 7.1 and 21 mg L^{-1}, respectively) were not significantly higher than in glacier-fed (*i.e.* 3.7, 0.5 and 6.8 mg L^{-1}, respectively) and rhithral streams (*i.e.* 11.9, 2.9 and 38.8 mg L^{-1}, respectively, Fig. 2, Supplementary Tab. 2). In addition, the difference may have been overlooked, since the power of the performed test (0.105) was below the desired values of 0.800, which implies a lower potential to detect differences where they actually exist. Concentrations of other ions (Na$^+$, K$^+$, Cl$^-$) were quite low and did not show significant differences in the surveyed water types (Supplementary Tab. 2).

Concentrations of the trace metals were highly variable, as shown in Fig. 3, but only in a few cases they exceeded the European limits for drinking waters (EU 83/1998, Supplementary Tab. 2). Sr, Al, Zn and Fe showed the highest median values (Fig. 3), which reached ~30 µg L^{-1} for Sr and Al, and a pronounced variability, while other metals, such as Ni, Mn and Ba, showed median values <3 µg L^{-1} but particularly high concentrations in some water samples (Supplementary Tab. 2). Concentrations of the majority of trace metals were very low in all the waters sampled in the AM sub-catchment, while the waters sampled in the MR sub-catchment showed higher concentrations (Supplementary Tab. 2). Permafrost-fed waters were enriched in some metals, particularly Sr, Zn and Ni, which reached the highest concentration in waters of the MR (*e.g.* up to 315 µg L^{-1} Sr in MR-RG2 and 60 µg L^{-1} Ni in MR-RG3) and SU sub-catchments (*e.g.* 410 µg L^{-1} Sr, 31 µg L^{-1} As, and 15 µg L^{-1} Ba in SU-RG2). On the contrary, Al, Mn and Fe showed higher concentrations in glacier-fed than in permafrost-fed waters in each sub-catchment, and reached their maximum in the glacial stream of AM (*i.e.* 211 µg L^{-1} Al and 250 µg L^{-1} Fe in AM-GL), and MR catchments (*i.e.* 255 µg L^{-1} Al and 80 µg L^{-1} Mn in MR-GL). The latter stream showed also the highest Zn concentration (*i.e.* 560 µg L^{-1}, Supplementary Tab. 2).

All surveyed waters were very poor in soluble phosphorus (median=2±1 µg L^{-1}) and ammonia concentrations (median=5±6 µg L^{-1}, up to 27 µg L^{-1} in the glacier-fed site MR-GL, Supplementary Tab. 2). All glacier-fed streams were characterised by outstanding concentrations of total phosphorous (≥70 µg L^{-1}), while concentrations of total nitrogen showed a strong decreasing gradient from the southernmost (4.8 mg L^{-1} in AM-GL) to the northernmost glaciers (only 0.08 mg L^{-1} in SU-GL1, Supplementary Tab. 2). Median concentrations of NO$_3$-N and SiO$_2$ were significantly lower in glacier-fed waters of all sub-catchments than in permafrost-fed and rhithral waters (One-way Anova test, P<0.01), while values in the permafrost-fed waters did not significantly differ from those of rhithral waters (Fig. 3). The highest NO$_3$-N and SiO$_2$ concentrations were detected in RG springs in the MR

sub-catchment (*i.e.* 1.4 mg L^{-1} and 7.8 mg L^{-1} in MR-RG3 and MR-RG4, respectively, Supplementary Tab. 2).

The median content of POM of the epilithic diatom samples collected from the surveyed waters was 25.9±25.5% of the dry mass (Tab. 1), with glacier-fed samples showing the lowest median content (9.3±2.5 %).

Permafrost-fed and rhithral waters showed a pronounced variability, with no evident relation to the presence of vegetation in the water bed or banks. Carbonate content was in general low (median=2.4±4.1 of the mineral ashes, Tab. 1), with lower values (≤2.93%) in the samples from all the glacier-fed and several permafrost-fed waters, and
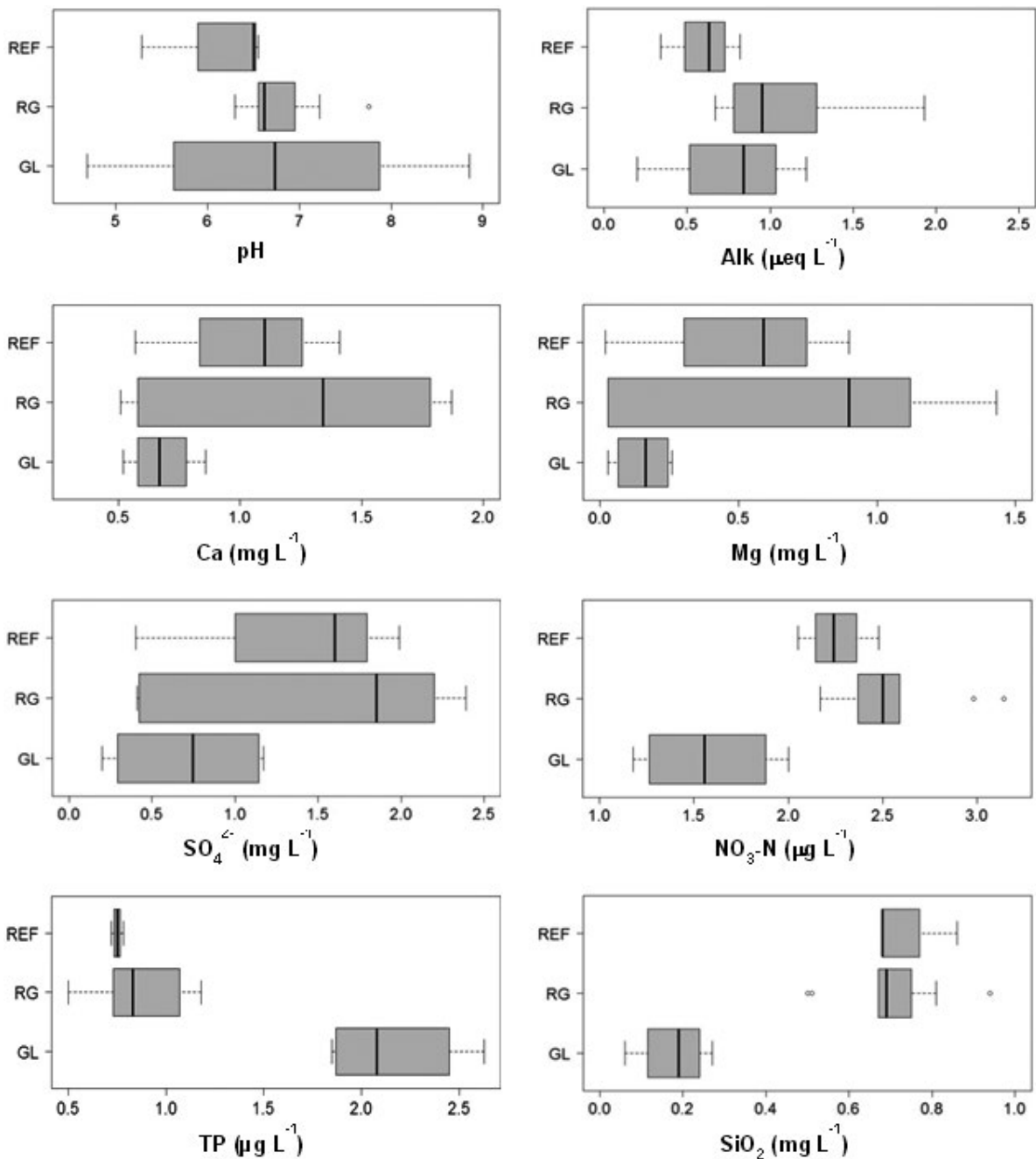

Fig. 2. Concentration ranges of selected water chemical variables measured in headwaters of different origin in the sub-catchments surveyed in the Italian Central Alps. All variables log-transformed (y=(logx+1)), except pH. Complete data are available in Supplementary Tab. 1. GL, glacier-fed; RG, permafrost-fed; REF, rhithral waters fed by precipitation and/or groundwater with no direct contact with glaciers or permafrost.

values exceeding 10% of the ash content only in the rhithral streams in the AM and MR sub-catchments (Tab. 1).

Diatom diversity and ecological classification

The taxonomical analysis of the 16 epilithic and 10 epiphytic samples collected in summer 2016 yielded a total of 120 diatom taxa. Epilithic diatom assemblages of all surveyed glacier-fed waters were extremely sparse, as only up to 42 valvae belonging to a few taxa (2-8) could be identified despite scanning the whole area of the slides (Supplementary Tab. 3.) Permafrost-fed and rhithral headwaters were characterized by denser diatom assemblages, which included several widespread taxa. The most common diatoms (*i.e.* reaching RA ≥0.5% in more than 10 samples) were *Achnanthidium minutissimum* var. *minutissimum* (Kützing) Czarnecki, *Diadesmis perpusilla* (Grunow) Mann, *Diatoma mesodon* (Ehrenberg) Kützing, *Psammothidium helveticum* (Hustedt) Bukhtiyarova Round and *P. daonense* (Lange-Bertalot) Lange-Bertalot, and *P. marginulatum* (Grunow) Bukhtiyarova and Round (Tab. 2). The most abundant taxon was the rheophilous *A. minutissimum* var. *minutissimum*, (maximum RA=78% in the rhithral stream A-REF-M, Tab. 2) which accounted to >30% of the total diatom abundance in the majority of the samples. Rare taxa, which reached RA ≥0.5% in only one sample, are listed in Supplementary Tab. 4.

The epilithic diatom assemblages of the permafrost-fed waters sampled in the MR sub-catchment showed lower number of taxa, and lower α-diversity (Shannon Index <2) and heterogeneity compared to permafrost-fed streams in the other sub-catchments (Shannon Index >2, Tab. 3). The sample collected from AM-RG4 showed the highest number of taxa (33), and the highest diversity and heterogeneity (Tab. 3). The epilithic diatom assemblages of the major reference streams of AM and MR sub-catchments were comparably diverse, while number of taxa and diversity were much lower in both the samples from MR-REF2 (Tab. 3), due to a very pronounced dominance of *P. helveticum* (RA=76%, Tab. 2). On average, the epiphytic diatom samples showed higher species richness (n =23) and diatom diversity (S.I.=2.5±0.1, Tab. 3) in comparison to the epilithic ones (n=16, S.I.=1.9±0.1, Tab. 3), with highest number of taxa and Shannon Index in the AM-RG4 sample and, secondarily, in the springs emerging from rock-glaciers in the SU Valley (Tab. 3).

According to the ecological classification of Van Dam *et al.* (1994), the majority (*i.e.* ~25%) of the diatom taxa identified prefers circumneutral waters and this category accounted in average to ~69% of the total diatom abundance in all the samples (Fig. 4a). In some samples with neutral pH values (*e.g.* MR-RG4 and SU-RG2) circumneutral taxa, such as *A. minutissimum sensu lato*, *D. perpusilla* and *D. mesodon*, were accompanied by higher proportions of alkaliphilus diatoms, such as *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot, *Amphora pediculus* (Kützing) Grunow (Fig. 4a), and *A. lineare* (W. Smith). Acidophilus taxa (*i.e.* *P. marginulatum*) represented the most abundant diatom category only in MR-RG3-E, while RA of the acidobiontic taxa *Eunotia exigua* (Brébisson ex Kützing) Rebenhorst exceeded 30% only in the epiphytic sample from MR-REF2 (Fig. 4a), in agreement with its quite acidic water pH (Supplementary Tab. 2). Diatom species found in all glacier-fed waters were mainly circumneutral or alkaliphilous, but this classification has to be considered as only indicative due to the very low number of diatom valvae identified in these samples.

Despite diatom taxa classified as indifferent to the water trophic status represent only ~11% of all identified taxa, in average they accounted to around 40% of the total diatom abundances in all the samples studied (Fig. 4b). According to the very low PO₄-P concentrations of all the surveyed headwaters, oligotraphentic taxa accounted to an average RA of around 22%. However, some mesotraphentic (*e.g. D. mesodon*) and even eutraphentic taxa (*e.g. P. lanceolatum* and *A. pediculus*) reached high relative abundance in some of the analysed epiphytic samples, such as AM-RG1-M, AM-RG3-M, and MR-RG4-M (Fig. 4b).

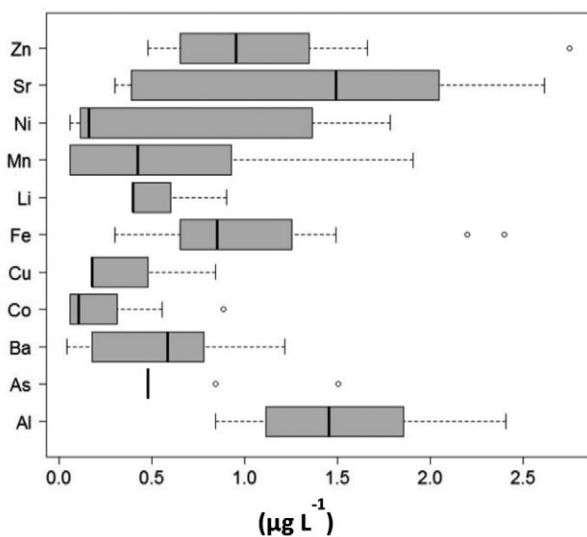Only around 10% of all identified diatom taxa were

Fig. 3. Log-transformed (y =(logx+1)) concentration ranges of the trace metals detected in headwaters of different origin in the sub-catchments surveyed in the Italian Central Alps. Complete data are in Supplementary Tab. 2. GL, glacier-fed; RG, permafrost-fed; REF, rhithral waters fed by precipitation and/or groundwater with no direct contact with glaciers or permafrost.

classified as occurring exclusively or mainly in water, while the majority (*i.e.* ~25% of the total) are considered as able to live also in places that are not permanently submerged (Fig. 4c). These tolerant taxa accounted to an average RA of ~47%, while aerophilous diatoms such as *D. perpusilla* and *P. marginulatum* were found especially in permafrost-fed springs with low water discharge (*e.g.* MR-RG1, MR-RG2, MR-RG3 and AM-RG3), where they showed RA between around 20 and 80% of the total diatom abundance. In general, the

Tab. 2. List of the 42 taxa used for the NMDS analysis (*i.e.* present with relative abundance ≥ 0.5% in at least two samples).

Taxa	Code	Nr	MRA	Sample	pH	TP
Achnanthidium minutissimum var. *minutissimum* (Kützing) Czarnecki	ADMI	18	78.14	AM-REF-M	cm	id
Diadesmis perpusilla (Grunow) D.G. Mann	DPER	17	80.78	AM-RG3-E	cm	o
Diatoma mesodon (Ehrenberg) Kützing	DMES	16	61.75	AM-RG3-M	cm	m
Psammothidium helveticum (Hustedt) Bukhtiyarova & Round	PHEL	14	76.01	MR-REF2-E	al	m
Psammothidium daonense (Lange-Bertalot) Lange-Bertalot	PDAO	14	11.85	MR-RG2-M	cm	o
Psammothidium marginulatum (Grunow) Bukhtiyarova & Round	PMRG	13	87.93	MR-RG3-E	ap	o
Encyonema silesiacum (Bleisch) D.G. Mann	ESLE	9	13.50	AM-RG3-M	cm	id
Encyonema minutum (Hilse) D.G. Mann	ENMI	8	4.10	SU-RG1-M	cm	n.a.
Psammothidium subatomoides (Hustedt) Bukhtiyarova & Round	PSAT	7	17.36	AM-RG1-E	ap	om
Psammothidium bioretii (H. Germain) Bukhtiyarova & Round	PBIO	7	4.55	AM-RG4-M	cm	m
Nitzschia perminuta (Grunow) M. Peragallo	NIPM	6	3.64	AM-RG4-M	al	om
Hannaea arcus (Ehrenberg) R.M. Patrick	HARC	6	2.83	MR-REF1-E	al	om
Meridion circulare (Greville) Agardh	MCIR	5	27.88	SU-RG2-M	al	id
Rossithidium petersenii (Hustedt) Round & Bukhtiyarova	RPET	5	15.48	SU-RG1-M	cm	o
Achnanthidium minutissimum var. *jakii* (Rab.) Lange-Bertalot & Ruppel	ADJK	5	7.93	MR-REF1-M	cm	n.a.
Amphora pediculus (Kützing) Grunow	APED	4	43.51	SU-RG2-E	al	e
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	EEXI	4	35.85	MR-REF2-M	ac	id
Psammothidium helveticum var. *minor* (Flower & Jones) Buczkó	PHMI	4	10.61	MR-REF2-M	al	m
Fragilaria rumpens (Kützing) G.W.F. Carlson	FRUM	4	3.64	AM-REF-M	cm	om
Fragilaria gracilis Østrup	FGRA	4	3.37	MR-REF1-M	cm	om
Achnanthidium lineare W. Smith	ACLI	3	38.74	SU-RG2-E	cm	n.a.
Reimeria sinuata (W. Gregory) Kociolek & Stoermer	RSIN	3	14.35	SU-RG1-E	cm	m
Amphora inariensis Krammer	AINA	3	6.97	SU-RG2-M	n.a.	o
Gomphonema parvulum (Kützing) Kützing	GPAR	3	6.01	SU-RG2-M	cm	e
Eunotia palatina Lange-Bertalot & Krüger	EPLT	3	3.54	MR-REF2-M	n.a.	n.a.
Denticula tenuis Kützing	DTEN	3	2.04	SU-RG1-M	al	m
Psammothidium sacculum (J.R. Kartner) Bukhtiyarova	PSAC	3	2.00	AM-RG4-M	n.a.	n.a.
Nitzschia alpina Hustedt	NZAL	3	1.84	AM-REF-E	n.a.	o
Undetermined pennate	UNPE	3	1.30	SU-RG2-E	n.a.	n.a.
Gomphonema pumilum var. *rigidum* Reichardt & Lange-Bertalot	GPRI	3	1.15	AM-REF-E	n.a.	id
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	PTLA	2	58.68	MR-RG4-E	al	e
Luticola mutica (Kützing) D.G. Mann	LMUT	2	4.56	MR-RG2-M	cm	e
Fragilaria vaucheriae (Kützing) J.B. Peterson	FVAU	2	4.20	MR-RG4-M	al	e
Fragilariforma virescens (Ralfs) D.M. Williams & Round	FVIR	2	3.30	MR-REF1-E	cm	om
Eunotia intermedia (Krasske ex Hustedt) Nörpel & Lange-Bertalot	EUIN	2	3.07	MR-REF1-E	ap	o
Adlafia suchlandtii (Hustedt) Monnier & Ector	ADLS	2	3.03	SU-RG2-E	n.a.	n.a.
Adlafia minuscula (Grunow) Lange-Bertalot	ADMS	2	2.96	MR-RG2-M	al	o
Staurosira venter (Ehrenberg) Cleve & J.D.Möller	SSVE	2	1.76	AM-RG4-E	al	me
Eunotia bigibba Kützing	EBIG	2	1.21	AM-RG1-M	ap	o
Cyclotella comensis Grunow	CCMS	2	0.97	MR-RG2-E	cm	n.a.
Stephanodiscus minutulus (Kützing) Cleve & Möller	STMI	2	0.75	AM-RG3-M	ab	he
Craticula molestiformis (Hustedt) Mayama	CMLF	2	0.61	SU-RG1-M	al	e

Code, taxon code according to Lecointe *et al.* (1993); Nr, number of sample where each taxon occurred with relative abundance > 0.5%; MRA, maximum relative abundance reached by each taxon in the sample set; Sample, diatom sample code for each surveyed headwater (as in Tab. 1); E, epilithic; M, epiphytic; pH and TP, preference for each diatom taxon for water pH and total phosphorus level, respectively, according to the classification proposed by Van Dam *et al.* (1994); ac, acidobiontic; ap, acidophilous; cm, circumneutral; al, alkaliphilous; ab, alkalibiontic; o, oligotraphentic; om, oligo-meso-traphentic; m, mesotraphentic; me, meso-eutraphentic; e, eutraphentic; he, hypereutraphentic; id, indifferent to P level; n.a., no classification available.

epiphytic samples showed a higher proportion of hydrophilic taxa (*e.g. P. daonense*, *Meridion circulare* (Greville) Agardh, *Encyonema silesiacum* (Bleisch) Mann, *D. mesodon*) compared to their epilithic counterparts (Fig. 4c, Tab. 2).

Diatom diversity and environmental variables

Due to the very low number of diatom valvae that could be found in the samples collected from the surveyed glacier-fed headwaters, only epilithic and epiphytic samples from permafrost-fed and rhithral headwaters (N=22) were considered for the NMDS ordination. A screen-plot analysis of the stress values of NMDS conducted with increasing number of dimensions indicated two dimensions (D1 and D2) as sufficient to represent the variability of diatom assemblages in this study. The two-dimensional NMDS explained 86.62% of the data variance with a stress value of 0.15, while NMDS analyses including more dimensions provided only slight improvements of both parameters.

The distribution of the samples within the NMDS space shows a partial grouping according to the water origin (*i.e.* permafrost-fed or rhithral). The samples from the two major rhithral streams (AM-REF and MR-REF1) are located close to the axis origin, and are accompanied by several epilithic and epiphytic samples from permafrost-fed headwaters (Fig. 5a). Samples from the smaller rhithral stream (MR-REF2) and from other permafrost-fed headwaters are instead located far away from the axis origin in the NMDS space, in relation to their high relative abundance of almost exclusive diatom taxa. In fact, both samples from the rock glacier-fed spring SU-RG2 showed high RA of *A. lineare* and *A. pediculus*, the epilithic sample from AM-RG3 and the epiphytic samples from the permafrost-fed waters AM-RG1, AM-RG3 and MR-RG2 showed particularly high abundance of *D. perpusilla* and *D. mesodon*, respectively (Fig. 5b). The epilithic sample from the permafrost-fed MR-RG3 showed high proportion of *P. marginulatum*, while both samples from MR-REF2 were dominated by varieties of *P. helveticum* and *Eunotia* spp. (Fig. 5b and Tab. 2). In contrast, all the samples grouped close to the axis origin were characterised by high relative abundances of *A. minutissimum sensu lato*, and, secondly, by *P. daonense*, several Fragilariaceae, *Nitzschia* spp., and *Gomphonema pumilum* var. *rigidum* Reichardt & Lange-Bertalot (Fig. 5b). The epilithic and epiphytic samples of the two major rhithral waters are located close to each other in the NMDS space, while are far from each other for all the permafrost-fed waters, with the sole exception represented by

Tab. 3. Species richness and α-diversity of epilithic and epiphytic diatom assemblages found in the headwaters surveyed in the AM, MR, and SU sub-catchments.

Sample	Taxa	S.I.±SD	Even	Hetr
SU-RG1-E	16	2.47±0.08	0.62	5.54
SU-RG1-M	27	3.03±0.09	0.64	8.18
SU-RG2-E	14	2.03±0.08	0.53	4.08
SU-RG2-M	23	2.69±0.08	0.60	6.47
MR-RG1-E	9	1.31±0.06	0.41	2.48
MR-RG2-E	10	1.72±0.07	0.52	3.29
MR-RG2-M	17	2.93±0.08	0.72	7.60
MR-RG3-E	8	0.70±0.08	0.23	1.62
MR-RG4-E	14	1.64±0.08	0.43	3.13
MR-RG4-M	16	2.20±0.08	0.55	4.58
MR-REF1-E	33	2.92±0.12	0.58	7.58
MR-REF1-M	33	2.88±0.01	0.57	7.37
MR-REF2-E	8	1.33±0.09	0.44	2.51
MR-REF2-M	11	2.22±0.07	0.64	4.64
AM-RG1-E	8	1.23±0.08	0.41	2.35
AM-RG1-M	16	2.25±0.09	0.56	4.77
AM-RG3-E	9	1.18±0.09	0.37	2.26
AM-RG3-M	22	2.19±0.11	0.49	4.55
AM-RG4-E	33	2.91±0.11	0.58	7.50
AM-RG4-M	41	3.17±0.11	0.59	8.98
AM-REF-E	31	2.87±0.11	0.58	7.33
AM-REF-M	21	1.57±0.11	0.36	2.96

Sample codes as in Tab. 2. Taxa, total number of taxa identified in each sample; S.I., Shannon Index; Even, evenness calculated on Shannon Index; Hetr, Hill's heterogeneity.

AM-RG4, and to a lesser extent by MR-RG4 (Fig. 5a). On the other hand, no sample grouping is evident according to their location in the different sub-catchments studied.

The vector fitting revealed highly significant correlations between periphytic diatom and the water variables associated to water acidity, *i.e.* pH and alkalinity (Tab. 4), and to concentrations of several trace metals. POM content of epilithic samples showed a nearly significant ($P<0.06$) relation. On the contrary, no significant relation emerged with the variables related to
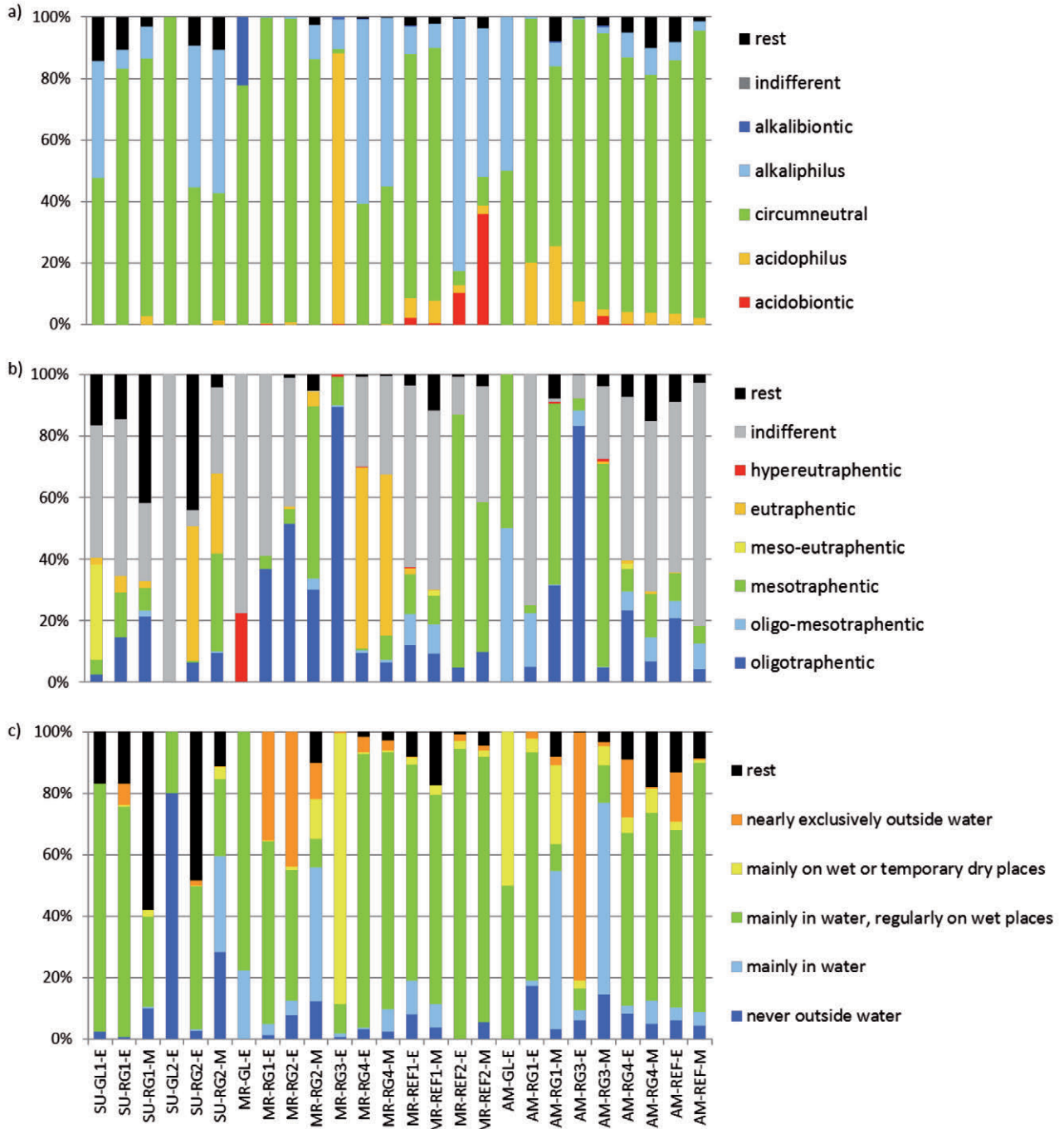

Fig. 4. Relative abundances of diatom taxa with different preferences for a) water pH, b) total phosphorus concentrations, c) moisture in the samples collected from the headwaters surveyed in the Italian Central Alps, according to the classification proposed by Van Dam *et al.* (1994). Sample codes as in Tab. 3.
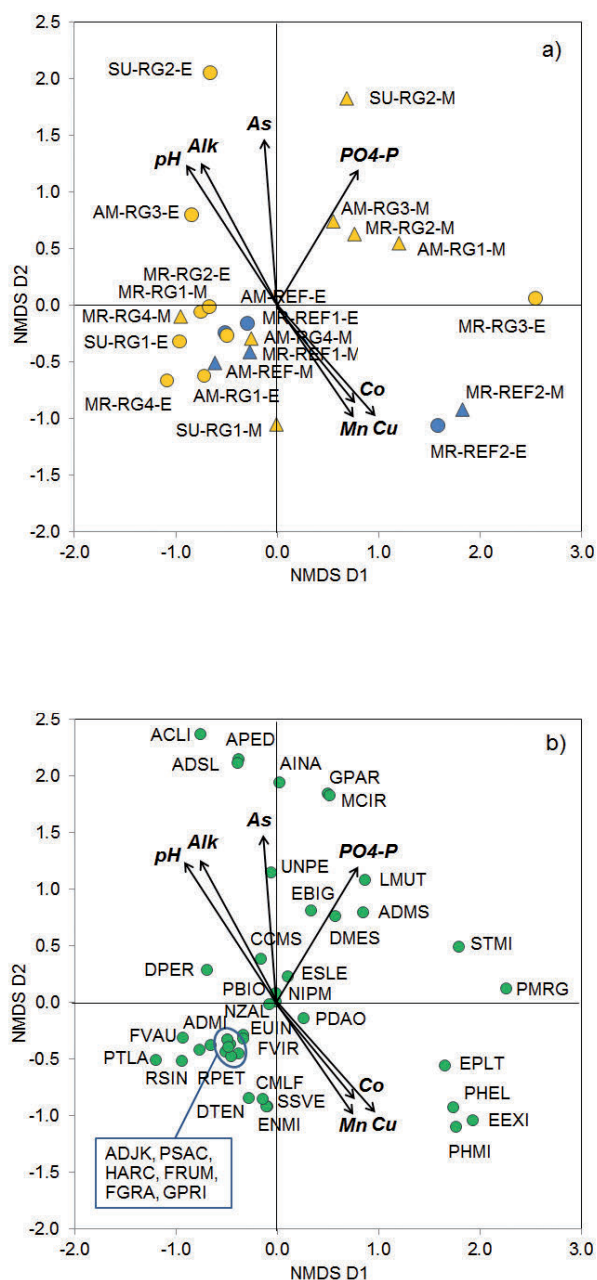
the water mineralization level, namely EC and major ions. The arrows of the acidity-associated variables point towards the positive side of NMDS dimension D2 (Fig. 5a), in agreement with the high pH and alkalinity of the headwater SU-RG2, while arrows associated to trace metals points to the negative region of NMDS D1, where samples characterized by lower pH and higher content of particulate organic matter in the epilithon are grouped. The location of the majority of the sample around the NMDS axis origin agrees with their intermediate chemical conditions (including nutrient level) independently from water type and sub-catchment (Fig. 5a).

Spearman rank correlation coefficients between sample scores on the NMDS dimensions and diatom-related variables (Tab. 3) confirmed the role of a few taxa (*i.e. D.*

Tab. 4. Significant (P<0.05) correlation coefficients between sample scores on NMDS dimensions D1 and D2 and chemical and habitat variables (based on the R vector fitting function, r^2), and diatom related variables (based on Spearman Rank correlation, ρ).

Variable	CC	P<	Dim
pH	0.51	**	n.a.
Alk	0.47	**	n.a.
HCO$_3$	0.46	**	n.a.
PO$_4$-P	0.44	*	n.a.
Cu	0.43	*	n.a.
As	0.46	**	n.a.
Co	0.33	*	n.a.
Mn	0.37	*	n.a.
Zn	0.26	0.07	n.a.
POM	0.23	0.08	n.a.
ADMI	-0.58	**	D1
DPER	-0.53	**	D1
DMES	0.56	**	D2
PDAO	0.82	***	D1
PHEL	0.61	**	D1
EEXI	0.45	*	D1
EPLT	0.47	*	D1
ac	0.62	*	D1
ap	0.50	*	D1
id	-0.42	*	D1
	-0.45	*	D2
ow	0.52	*	D2
mw	-0.67	***	D1
	-0.46	*	D2
ws	0.87	***	D1
ms	-0.52	*	D1

CC, correlation coefficient; Dim, NMDS dimension D1 or D2; n.a., not available. Diatom codes as in Tab. 2; diatom preferences for pH, TP and moisture as in Tab. 2; ow, occurring almost exclusively in water; mw, occurring mainly in water; ws, mainly occurring in water but frequent also on wet substrata; ms, mainly occurring on wet or temporarily dry substrata; ***P<0.001; **P<0.01; *P<0.05.

Fig. 5. NMDS ordination of a) the diatom samples collected from the surveyed headwaters based on relative abundance of epilithic and epiphytic diatom taxa reaching relative abundance ≥0.5% in at least two samples; b) species distributions in the ordination space. The vector fitting is restricted to environmental variables significantly correlated (P≤0.05) with the sample ordination. Vector orientation indicates the direction of the most rapid change for each variable, vectors lengths indicate the correlation between the variable and the sample ordination. Blue circles and triangles, epilithic and epiphytic samples, respectively, from rhithral headwaters fed by precipitation and/or groundwater; yellow circles and triangles, epilithic and epiphytic samples, respectively, from permafrost-fed headwaters. sample and species codes as in Tabs. 2 and 3.

perpusilla, *D. mesodon*, *P. helveticum, E. exigua, Eunotia palatina* Lange-Bertalot and Krüger) in determining the separation of several samples from permafrost-fed waters along both NMDS D1 and D2, from the most numerous group of samples, which were characterized by the dominance of common taxa, especially *A. minutissimum* var. *minutissimum* and *P. daonense* (both correlated to D1, Tab. 4). The correlation analyses revealed also a significant positive correlation between samples scores on D1 and the cumulative abundance of acidophilous and acidobiontic taxa, while taxa indifferent to the water phosphorus level showed a negative correlation with the same NMDS dimension (Tab. 3). Furthermore, samples score along NMDS D1 are correlated positively with the abundance of taxa occurring both in water and on moist soil, and negatively with taxa occurring mainly in water or on soil, respectively (Tab. 3). Sample scores on D2 are correlated positively to taxa occurring only in water and negatively to taxa occurring mainly in water, *i.e.* with scarce tolerance to desiccation (Tab. 3).

DISCUSSION

Research on alpine rock glaciers has recently gained attention as part of the mountain cryosphere that is increasingly threatened by the global climate warming (IPCC, 2013). However, the knowledge on the impact of climate change on permafrost thawing processes, and, consequently, on the chemical and biological quality of headwaters emerging from Alpine rock glaciers is still scarce (Colombo *et al*., 2018). Despite the limit posed by the small number of headwater surveyed, the present study confirms the results obtained by previous investigations on chemical features and biodiversity of permafrost-fed headwaters waters in the Central Alps (Thies *et al*., 2007, 2013, 2018; Ilyashuk *et al*., 2014; Lösch *et al*., 2015; Thaler *et al*., 2015), and provides new insight on aspects scarcely considered so far.

Similarly to what observed by the mentioned studies, the permafrost-fed springs and streams surveyed in each of the sub-catchments studied in this investigation showed higher EC values and elevated concentrations of Ca^{2+}, Mg^{2+} and SO_4^{2-} in comparison to rhithral headwaters with no contact with permafrost. The highest EC values detected in the present study (~500 μS cm^{-1}) are comparable with those found in other catchments on crystalline bedrock of the Central Alps (Krainer *et al*., 2011; Thies *et al*., 2007, 2013; Lösch *et al*., 2015) and agree with the increasing observations that waters emerging from rock glaciers are characterized by higher solute concentrations (Colombo *et al*., 2018). On the opposite, the glacier-fed streams sampled in each sub-catchment were characterized by low mineralization, as commonly observed in glacial waters of siliceous

catchments worldwide (Slemmons *et al*., 2013). All headwaters surveyed in this study were very poor in soluble phosphorus, which likely represents a limiting factor for diatom growth, while concentrations of particulate TP and TN were particularly high in the glacier-fed streams, in agreement with previous studies of kryal waters (Hodson *et al*., 2004; Rott *et al*., 2006). The small number of studied sub-catchments does not allow understanding whether the higher TN concentrations detected in the southernmost headwaters may be due to N deposition, owing to their closeness to the atmospheric sources in the Po plain, or whether they are casual or related to geomorphological features. The organic content of epilithic biofilms appears to be independent from both water type and catchment geology, as it is likely related to local factors affecting the algal development, such as water turbidity and turbulence.

The studied permafrost-fed waters represent a quite heterogeneous group in comparison to glacier-fed and rhithral waters. In fact, all waters on the intrusive bedrock of the AM Valley were characterised by lower EC and ion concentrations than the waters on the metamorphic bedrock of the MR and SU catchments. This suggests that the chemical fingerprint of waters of different origin becomes less clear when considering catchments with different geology. Also the difference between permafrost-fed and rhithral waters is not as neat as observed in other areas of the Central Alps (Thies *et al*., 2007, 2013), which focused on geologically homogeneous watersheds, where chemical differences due to water origin are maximized. A strong geological control of type and amount of weathering products in headwaters has been outlined also in an extensive study on glaciated watershed in western USA (Fegel *et al*., 2016).

The double control exerted by water origin and watershed lithology over the concentrations of solutes in Alpine headwaters is evident also when considering trace metals. In fact, concentrations were very variable from site to site, but in general lower in the waters of AM catchment, and higher in permafrost-fed waters. This supports the hypothesis that trace metals of geological origin can become concentrated in rock glacier outlets due to a set of physical and chemical processes operating in permafrost, such as enrichment related to higher water residence time (Esposito *et al*., 2016) and different groundwater paths (MacLean *et al*., 1999; Frey and McClelland, 2009), freezing-thaw cycles (Williams *et al*., 2006; Thies *et al*., 2007, 2013), or acid rock drainage (ARD) induced by sulphide oxidation on freshly exposed rocks (Todd *et al*., 2012). In addition, the fact that only Al, Mn and Fe reached higher levels in glacier-fed than in permafrost-fed waters in each catchment agrees with recent observations that the mentioned processes are not exclusive of permafrost, but can occur also in other high-

altitude systems in relation to climate warming (Zaharescu *et al.*, 2016). The present study did not provide further elements on the possible mechanism controlling trace metal enrichment in Alpine headwaters, as no relation was found with possible regulating factors, such as water pH or particulate matter. However, this study suggests that, although the measured concentrations of trace metals exceed the European guidelines for drinking waters only in a few cases, they potentially represent a threat at local level (*e.g.* for Alpine huts and cattle) in the predicted future context of reduced glacier mass and atmospheric precipitation.

The overall diversity of epilithic diatoms in the surveyed headwaters was somewhat lower with respect to previous studies (Thies *et al.*, 2013; Lösch *et al.* 2015), in relation to the low species richness and pronounced dominance by a single or a few taxa in several permafrost-fed water, especially in the MR sub-catchment. In general, the rhithral waters showed much higher α-diversity, although the highest number of species and Shannon diversity was found in a water classified as permafrost-fed (*i.e.* AM-RG4-E). The reason for the high diatom diversity of this sample is not clear, but the fact that this headwater emerges from a talus slope, which may contain no ice according to the probabilistic model for permafrost distribution in the Alps (Boeckli *et al.*, 2012), might explain the similarity between the diatom composition of AM-RG4-E and AM-REF-E (see below). Glacier-fed waters surveyed in this study were characterized by extremely scarce diatom assemblages, in agreement with previous investigations in the Alps, Tatra and Himalaya (Uehlinger *et al.*, 1998; Cantonati *et al.*, 2001; Rott *et al.*, 2006; Kawecka *et al.,* 2012; Alber, *personal communication*), but partially in contrast with results from other mountain regions (Gesierich and Rott, 2012). In the glacier-fed headwaters surveyed in the present study diatom growth appears to be principally limited by physical constraints typically characterizing kryal waters, such as channel instability, reduction of light penetration and scorching exerted by the high mineral turbidity, which reset the diatom succession and reduce taxonomic richness (Bona *et al.* 2012; Fell *et al.*, 2018).

When excluding the outliers represented by glacier-fed waters, the differences between epilithic diatom communities in permafrost-fed and rhithral waters appears to be reduced in comparison with chemical differences. The multivariate analysis outlined that diatom assemblages of around one half of the permafrost-fed samples are highly comparable to those of their rhithral counterparts. These samples were characterized by intermediate chemical conditions, including a moderate to low trace metal concentrations, and their diatom assemblages were correspondingly dominated by the widespread reophilous and tolerant *A. minutissmum* var.

minutissmum, which was accompanied by a set of subdominant species that are typical of oligotrophic, poorly mineralized, circumneutral to slightly acidic arctic-alpine or high altitude freshwaters on crystalline bedrock, such as *D. mesodon*, *D. perpusilla*, *P. daonense*, and several fragilarioid and cymbelloid taxa (Cantonati *et al.*, 2001; Rott *et al.*, 2006; Rimet *et al.*, 2007; Gesierich and Rott, 2004, 2012). Interestingly, diatom assemblages of the two rhithral streams sampled in the AM and MR sub-catchments presented very similar diatom assemblages, despite the much higher mineralization level of the second stream. However, the two waters showed identical pH, which is known as one of the key drivers for benthic diatoms in Alpine headwaters (Cantonati *et al.*, 2001, 2012; Tolotti, 2001; Marchetto *et al.*, 2009), and were characterized by similar habitat conditions (*e.g.* channel and surrounding vegetation, water turbidity and velocity). This stresses the need, when comparing the biodiversity of Alpine headwaters, of a holistic evaluation, that considers, beside water chemistry, also the habitat template and the temporal variability of physical constraints, as suggested by Uehlinger *et al.* (2010), Sertić Perić *et al.* (2015) and Feret *et al.* (2017).

The epilithic assemblages of the remaining permafrost-fed waters differed from those of rithral samples, mainly in relation to high abundances of single or a few taxa, that in turn appeared to be related to peculiar water characteristics. The samples with the most peculiar diatoms assemblage were separated from the group of "intermediate samples" according first to their pH and alkalinity, and secondly to the concentration of some trace metals. Alkaliphilous taxa (such as *A. lineare*) were dominant in SU-RG2-E, which showed also the highest concentration of As, Ba and Sr, while a set of acidophilous taxa (such as *P. helveticum,* and *Eunotia* spp.) were dominant at the other pH extreme, *i.e.* in samples that also showed the highest concentrations of Co, Cu, Mn and Al. The sample MR-RG3-E represents an outlier, as, despite its slightly acidic pH, it showed high concentration of several metals, especially Ni, and was dominated by the acidobionthic *P. marginulatum*.

These results confirm previous evidences of the relation between diatom species composition of Alpine headwaters fed by thawing permafrost and heavy metal concentrations (Thies *et al.*, 2013; Lösch *et al.*, 2015), although trace metal concentrations in the surveyed waters were not so high as in other catchments studied in the Central Alps (Thies *et al.*, 2007, 2013). Unlike in these previous studies, no univocal negative effect of trace metals on diatom diversity could be pointed out, since the reduced values of Shannon index and heterogeneity of the samples from permafrost-fed waters appeared to be independent from the trace metal levels. In addition, all the "separated" samples were collected in springs or

streams with low water discharge and velocity, and showed, accordingly, high proportion of taxa that can tolerate periods of desiccation. This again stresses the importance of habitat features in regulating diatom biodiversity of Alpine headwaters (Rott *et al*., 2006).

Compared to previous investigations, the present study provides first information also on the epiphytic diatom assemblages of Alpine headwaters fed by permafrost, which, in general, were more diverse than epilithic assemblages in all water types. This higher diversity was not due to the presence of typically epiphytic taxa (such as *M. circulare*), but to the presence of higher proportion of rare taxa, such as several *Encyonema*, *Gomphonema* and *Nitzschia* species, and *Denticula tenuis* Kützing. In addition, the multivariate analysis revealed that epilithic and epiphytic diatom assemblages were very similar in rhithral streams, while they were quite different in all the permafrost-fed headwaters. The sole exception is represented by the AM-RG4 samples, which showed several characteristic similar to a rhithral water, as mentioned above, and to a lesser extent by MR-RG4. The difference between epilithic and epiphytic diatoms is not easy to be interpreted based on the chemical and habitat data collected in the present study. However, the aquatic mosses are likely able to provide a favourable physical habitat for diatom growth, and to smooth the effects of chemicals, such as trace metals. These aspects surely deserve to be investigated more in detail in the future.

CONCLUSIONS

The present study contributes to the chemical and biological characterisation of high mountain freshwaters in the Italian Alps, with a particular focus on waters fed by permafrost. The results support previous observations outlining the impact of thawing permafrost on the chemistry of headwaters emerging from active rock glaciers, in particular as regards solute content and trace metal concentrations. However, this study clearly indicates that the lithology of the watershed also plays a crucial role in determining the chemical features of headwaters on different bedrocks. In addition, the present study confirms the pivotal importance of pH in modulating the epilithic and epiphytic diatom assemblages of permafrost-fed waters, as well as the capacity of trace metals to negatively affect diatom diversity and to select tolerant diatom species in these waters. On the contrary, the other chemical variables seem to play a subordinate role, despite the high variability of electrical conductivity and major ion concentrations found in the surveyed waters.

The comparison of chemistry and diatom diversity in waters with different origin, *i.e.* fed by glacial melt water, permafrost thawing, and precipitation/groundwater,

provides useful information to predict the possible future changes in the physical chemical and biological features of Alpine headwaters in relation to the progressing climate-related glacial retreat. In particular, differently to what predicted for other elements of the biota, especially macroinvertebrates (Khamis *et al*., 2014), the diatom α-diversity of kryal headwaters does not seem to be threatened by the glacier retreat. On the contrary, kryal diatom assemblages are expected to become more diverse thanks to the improving environmental condition (*i.e.* lower physical and chemical shear stress). On the other side, permafrost-fed waters are characterized by a lower diatom α-diversity of the biota, mainly in relation to their enrichment in trace metals, but possibly also to limiting physical habitat conditions, such as low discharge. This suggests that permafrost-fed waters might play a moderate role as biodiversity refugia (Hotaling *et al*., 2017) for benthic microalgae in a warmer future with reduced precipitations. On the contrary, a homogenization and banalization of the periphytic diatom assemblages at both headwater and regional level (*i.e.* loss of β- and γ-diversity) can be predicted for the future, due to both the selection of a few tolerant taxa (Fell *et al.*, 2018) and to the reduction of habitat variability. Within this context, the wide variability of Alpine headwaters in relation to water origin, watershed lithology and individual habitat features, seems to have a larger potential in preserving the high-altitude biodiversity. We advocate an increase in the number of case studies across habitats, seasonal aspects and biological groups, in order to improve the understanding the processes affecting diatom diversity in high altitude waters of different origin, and to provide more robust and generalized assessment of the ecological role of these aquatic habitats in the next decades.

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