



# Diversity and Ecology of Chlorophyta (Viridiplantae) Assemblages in Protected and Non-protected Sites in Deception Island (Antarctica, South Shetland Islands) Assessed Using an NGS Approach

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## Abstract

Assessment of the diversity of algal assemblages in Antarctica has until now largely relied on traditional microbiological culture approaches. Here we used DNA metabarcoding through high-throughput sequencing (HTS) to assess the uncultured algal diversity at two sites on Deception Island, Antarctica. The first was a relatively undisturbed site within an Antarctic Specially Protected Area (ASPA 140), and the second was a site heavily impacted by human visitation, the Whalers Bay historic site. We detected 65 distinct algal taxa, 50 from within ASPA 140 and 61 from Whalers Bay. Of these taxa, 46 were common to both sites, and 19 only occurred at one site. Algal richness was about six times greater than reported in previous studies using culture methods. A high proportion of DNA reads obtained was assigned to the highly invasive species *Caulerpa webbiana* at Whalers Bay, and the potentially pathogenic genus *Desmodesmus* was found at both sites. Our data demonstrate that important differences exist between these two protected and human-impacted sites on Deception Island in terms of algal diversity, richness, and abundance. The South Shetland Islands have experienced considerable effects of climate change in recent decades, while warming through geothermal activity on Deception Island itself makes this island one of the most vulnerable to colonization by non-native species. The detection of DNA of non-native taxa highlights concerns about how human impacts, which take place primarily through tourism and national research operations, may influence future biological colonization processes in Antarctica.

**Keywords** Antarctica · Green algae · High-throughput sequencing · South Shetland Islands

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## Introduction

Deception Island, located in the South Shetland Islands, with a maximum altitude of 576 m a.s.l., is one of very few active volcanoes in the Antarctic Treaty area (above latitude 60° S) and one of only two in the region that has had human-witnessed eruptions [1]. It is a relatively young volcanic island, with an age less than 100 kya [2] and is still undergoing colonization. Deception Island is horseshoe-shaped with a diameter of ca. 15 km. Its inner flooded caldera forms Foster Bay, and about 57% of its land surface is currently covered by ice [1, 3]. Two national Antarctic research stations are presently active on the island (the Argentinean Decepción and Spanish Gabriel de Castilla), and historically Chile and the UK also operated stations on the island. The Chilean station was destroyed and the British station heavily damaged during the most recent series of eruptions on the island in the late 1960s [3]. Whalers Bay (62° 59' S, 60° 34' W) was also the

site of an active whaling station in the early twentieth century, and the station's remains are now designated as a historic site. Whalers Bay is also one of the most visited sites in Antarctica by tourists, with more than 80,000 tourists visiting the island in the summers of 2007–2010 [4]. The unique geology, history, biota, and esthetic values, as well as the activity of multiple national operators, underlie the designation of the entire island as an Antarctic Specially Managed Area (ASMA 4). In addition, Deception Island includes two Antarctic Specially Protected Areas (ASPAs), designated as ASPA 140 (terrestrial, formed of multiple subsites) and 145 (marine).

Terrestrial biological communities on Deception Island have received some research attention. The flora includes a number of Bryophyta (57 species) [5] and Marchantiophyta (6 species) [6], some associated with geothermally heated sites [3] and not found elsewhere in the Antarctic Treaty area, though also occur in similar geothermal habitats on the maritime Antarctic South Sandwich Islands [7, 8]. No flowering plants are cited in the management plan for the island [9], but both native Antarctic species are known to occur. Similarly, these sites also host a number of terrestrial micro-arthropods not found elsewhere in the treaty area [10–12]. As well as having specific geothermally active sites, the entire island is thought to be under slight geothermal influence and is thought to be particularly vulnerable as a result to the colonization (by natural means or human-assisted) of non-native species of plants and invertebrates [11, 13].

Traditionally the study of soil algal diversity in Antarctica has relied heavily on morphology alone, including two methods, direct observation using microscopy and cultivation [14–20]. Soil microalgal assemblages on Deception Island were investigated by Fermani et al. [17], who sampled across 18 sites that reflected the island's geological and edaphic variability and reported the presence of 140 taxa, of which 26 were representatives of Chlorophyta. However, due to the extreme environmental conditions typical of Antarctica, microalgae and other taxa can exhibit significant morphological variation due to local environmental conditions [21]. In addition, cultivation methods can be selective towards generalist rather than rare or specialist species [15], with some species simply not being currently cultivable. Thus, traditional culturing methods fail to adequately represent the range of microalgal diversity in nature [22, 23].

Recent developments in molecular biology have allowed considerable advances in the assessment of microbial diversity in environmental samples such as soil and water. DNA metabarcoding using high-throughput sequencing (HTS) represents a superior method for detection of rare species [23, 24], including their resting stages which are typically not detected in morphological surveys [25]. To date very few studies exist comparing morphological and molecular approaches for Antarctic algae. Rippin et al. [24] in a study on Livingston Island suggested that a molecular approach yielded richness

estimates 11 times higher than a traditional morphological approach. Fraser et al. [26], working on continental Antarctica volcanoes, investigated the importance of geothermal areas for supporting life, including their possible role as refugia during glaciations. Garrido-Benavent et al. [27], also working on Livingston Island, used a more ecological approach to investigate the successional patterns of microorganisms, comparing assemblages of bacteria, fungi, and algae present in three different substrata (moraines, rocks, and soil). In the current study, we applied HTS for the first time to investigate Chlorophyta (Viridiplantae) diversity in assemblages present in soil samples from two sites on Deception Island, one from within a protected area (ASPA 140 subsite B) and the other under considerable visitor impact.

## Materials and Methods

### Taxonomic Definitions

We follow the definition of Viridiplantae of Leliaert et al. [28], as a monophyletic group comprising Chlorophyta and Streptophyta. Only green algae lineages are considered here, regardless of their systematic classification. We do not discuss the taxonomy of groups cited or nomenclatural validity, and geographical distributions are based on cited literature and Guiry and Guiry [29]. Taxa are referred to at genus and species level and higher hierarchical levels (e.g., families, orders) are omitted.

### Study Sites and Sample Collections

Deception Island is located at 62° 57' S and 60° 38' W (Fig. 1). Two sites were selected to assess and compare the diversity of soil green algal communities on the island. The first was within a protected area (ASPA 140, subsite B, Crater Lake) (Fig. 1), which has a low degree of disturbance by both volcanic and human research activity (tourists are prohibited from entering). The area is distant from the shore, slightly raised elevation, and protected from direct exposure to sea spray by coastal hills. The second area was in Whalers Bay, which lies within the ASMA but beyond the limits of ASPA 140 and is a less protected and much more intensively visited site, both by tourists and national operator personnel, and was also more directly impacted by eruptions in the late 1960s [1, 3, 17]. The area is at sea level and its location close to the shore is more susceptible to sea spray.

During the austral summer of 2018/2019, soil samples (ca. 250 g each) were collected in both sites using a sterilized spatula and avoiding visibly vegetated areas (bare soil). Samples were kept in sealed sterile plastic bags (Whirl Pack®/US) and frozen (–20 °C) until DNA extraction. Seven (non-composite) samples from each site were collected

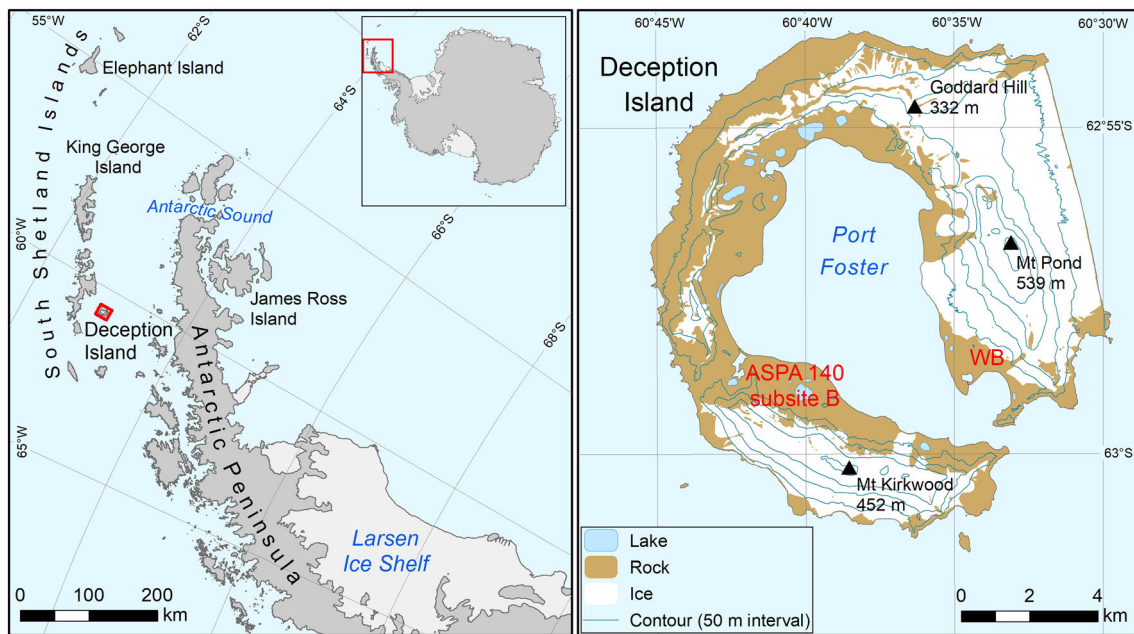


Fig. 1 Maps showing location of Deception Island and of the two sampled sites, ASPA 140 subsite B and Whalers Bay (WB)

from the upper 5 cm of the soil profile for use in DNA studies, totaling 14 samples [30–32].

## DNA Extraction and Data Analysis

### DNA Extraction, Illumina Library Construction, and Sequencing

Total DNA was extracted from environmental samples using the QIAGEN Power Soil Kit (QIAGEN, Carlsbad, USA), following the manufacturer's instructions. DNA quality was analyzed by agarose gel electrophoresis (1% agarose in 1 × Trisborate-EDTA) and then quantified using Quanti-iT™ Pico Green dsDNA Assay (Invitrogen). The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification [33, 34] using the universal primers ITS3 and ITS4 [35]. Library construction and DNA amplification were performed using the Library kit Herculese II Fusion DNA Polymerase Nextera XT Index Kit V2, following Illumina 16S Metagenomic Sequencing Library Preparation Part #15044223 Rev. B protocol. Paired-end sequencing (2 × 300 bp) was performed on a MiSeq System (Illumina) by Macrogen Inc. (South Korea).

### Data Analysis

Raw reads were interleaved and filtered using BBDuk version 38.34 (BBMap—Bushnell B.—[sourceforge.net/projects/bbmap/](http://sourceforge.net/projects/bbmap/)) to remove Illumina adapters, known Illumina artifacts, and PhiX Control v3. Quality read filtering was carried out using Sickle version 1.33 [36] to trim ends 3' or

5' with low Phred quality score, and sequences smaller than 100 bp were also discarded. Remaining fastq sequences (Supplementary Table 1) were converted to FASTA format using reformat.sh from BBMap and compared against Viridiplantae ITS2 database (<http://its2.bioapps.biozentrum.uniwuerzburg.de/>) [37] using Nucleotide-Nucleotide BLAST 2.6.0+. Taxonomic assignments were determined by the maximal matching from BLASTn alignment using MEGAN V6.12.3 [38].

### Ecological Diversity Analysis

Rarefaction calculations were carried out using the rarefaction analysis command in the software MOTHUR, where we clustered sequences into OTUs by setting a 0.03 distance limit. The following diversity statistics were calculated: Fisher's  $\alpha$ , Shannon, Margalef, Simpson, and evenness, to assess alpha diversity. We also performed a diversity  $t$  test, for comparison of the Shannon and Simpson diversities in both areas using PAST 3.26 [39]. Non-metric multidimensional scaling (NMDS) ordination was performed to check the existence of differences in community composition at the sampled localities, using the Bray-Curtis index and also using PAST 3.26 [39]. For comparison between the two communities in relation to turnover and nestedness, we calculated beta diversity indexes using “beta-multi” option in the R package “betapart” using the Sørensen dissimilarity index [40]. As there are no studies available using the same approach, we used two lists of Chlorophyta extracted from publications by Broady [41, 42] in our statistical analyses for exploratory comparisons. Venn diagrams are based on Bardout et al. [43]

## Results

A total of 4,097,406 reads were generated in the sequencing run, and 3,608,576 ITS2 reads remained after quality filtering (Suppl. Table ST1, Suppl. Fig. S1). The use of 97% similarity cutoff [44, 45] resulted in 67 ITS2 OTUs, representing 65 taxa of soil green algae according to the BLAST search. The calculated rarefaction curves approached a plateau, indicating that the reads gave an accurate representation of the local algal diversity in both sites (Suppl. Figs. S2 and S3).

Of the overall total of 65 taxa of soil green algae, 50 were present at ASPA 140 subsite B (Crater Lake) and 61 at Whalers Bay. These included 35 taxa previously unreported from Antarctica (Table 1). Abundance and distribution are shown in Table 2. The alpha diversity indices are presented in Table 3. The diversity  $t$  test was significant ( $p < 0.05$ ), indicating differences in species composition between the two assemblages.

Alpha diversity indexes are presented in Table 3. The Shannon index values differed significantly between Crater Lake and Whalers Bay, with diversity being higher in Whalers Bay and that species assemblages differing between the two areas. The Simpson index was high in both Crater Lake and Whalers Bay and again significantly different. Equitability  $J$  (Pielou's evenness) had values close to 0.6, suggesting that both species assemblages did not have similar patterns of abundance. Together, these indexes confirm that alpha diversity of these locations, even though numerically similar, were heterogeneous.

NMDS graphs (Suppl. Fig. S4), with stress value 0.09, and first and second axes explaining 80% and 17% of overall variation suggest little variation between the two assemblages, and both are equally heterogeneous. Beta diversity comparisons showed a dominance of the turnover component ( $\beta_{sim}$ ) for both assemblages (Table 4), suggesting that disturbances in the areas did not influence beta diversity.

## Discussion

The present study corroborates previous findings that DNA metabarcoding using HTS is more effective in detecting soil microalgal diversity than traditional methods of cultivation or direct observation. For the two study sites examined here, ASPA 140 subsite B and Whalers Bay, algal richness detected by this means was about six times higher (65 taxa) than reported by Fermani et al. [17] from the same two locations based on cultivation (nine taxa). Consequently, the overlap in species diversity is high when comparing the two studies, as well as between sites based on the molecular data, since 46 of the 65 taxa (ca. 70%) were found in both ASPA 140 and Whalers Bay (Fig. 2). Of the taxa reported, *Raphidonema nivale*

was the only one detected in both sites in both studies. About 53% (35 out of 65) taxa have not been previously recorded in Antarctica (Table 1), with previous records of some from areas remote from Antarctica (e.g., *Caulerpa webbiana*). We stress that detection of DNA does not confirm presence of the most similar organism whose sequence data are available in databases [46]; it means that a sample was somehow exposed to the organism or parts of it, even single cells a factor that needs to be taken into account when using molecular data to inform on conservation or management decisions, for instance when dealing with invasive species [47].

It is known that many factors, including extraction methodology, PCR, and primer bias, can affect the number of reads [25] and thus could lead to misinterpretation of abundance [48]. However, Giner et al. [49] concluded that such biases did not affect the proportionality between reads and cell abundance, implying that more reads are genuinely linked with higher abundance [50, 51]. Therefore, we used the number of reads as a proxy for relative abundance and for comparative purposes only.

The calculated ecological indices indicate that both study sites hosted moderate algal diversity (Fisher's  $\alpha$ ) and richness (Margalef) with low dominance (Simpson's), with Whalers Bay being more diverse than ASPA 140 subsite B. In the absence of comparable published studies of molecular diversity of Antarctic algae against which to compare our data, we used data extracted from the studies of Broady [41, 42] (Table 3) using culture approaches, while accepting that the latter study sites are remote from Deception Island in Antarctica and not closely similar habitats. The use of HTS unveiled a cryptic algal community present in the soils of Deception Island which was more diverse, richer, and with low dominance when compared with Broady's studies [41, 42], but we accept that the latter study sites are much further south (at least 15° of latitude) and also at around 3000 m a.s.l. Recent studies by Fraser et al. [17] and Garrido-Benavent et al. [27] had distinct aims and used different taxonomic ranks (genus and order, respectively), reducing the ability to make direct comparisons with the current study. However, comparing diversity data across studies (Table 4), the Shannon and Simpson indices differed little in the microalgal assemblages of ASPA 140 and Whalers Bay between the current study and those calculated by Garrido-Benavent et al. [27], although evenness was higher in the latter study. Garrido-Benavent et al. [27] described similar overall patterns for Chlorophyta, suggesting that local disturbances had not affected the assemblage composition.

Given the lack of modern molecular studies of the diversity of Antarctic microalgal communities, it is likely that many taxa reported for the first time from Antarctica, as in the data

**Table 1** Taxa present in soil samples at two sites on Deception Island: ASPA 140 subsite B (ASPA 140) and Whalers Bay (WB)

| Taxa   | ASPA 140<br>HTS | WB<br>HTS | ASPA 140<br>Cult | WB<br>Cult |
|--|-----------------|-----------|------------------|------------|
| <i>Bryopsidales</i>  |                 |           |                  |            |
| <i>Caulerpa webbiana</i> Montagne*   | x               | x         |                  |            |
| <i>Chlamydomonadales</i>   |                 |           |                  |            |
| <i>Chlamydomonas nivalis</i> (F.A. Bauer) Wille                                    | x               | x         |                  |            |
| <i>Chlamydomonas proboscigera</i> Korshikov*                                       | x               | x         |                  |            |
| <i>Chlamydomonas raudensis</i> Ettl.   |                 | x         |                  |            |
| <i>Neochlorosarcina negevensis</i> (Friedmann & Ocampo-Paus) Shin Watanabe*        | x               | x         |                  |            |
| <i>Neosporangiococcum cf. alabamense</i> (Deason) Deason                           |                 |           | x                |            |
| <i>Neosporangiococcum vacuolatum</i> Deason & Cox                                  |                 |           | x                |            |
| <i>Sanguina nivaloides</i> Procházková, Leya & Nedbalová                           |                 | x         |                  |            |
| <i>Tetracystis diplobionticoidea</i> (Chantanachat & Bold) P.A. Archibald & Bold*  |                 | x         |                  |            |
| <i>Tetracystis vinatzeri</i> Ettl & Gärtner*                                       | x               | x         |                  |            |
| <i>Tetracystis</i> sp.   | x               |           | x                |            |
| <i>Chlorellales</i>  |                 |           |                  |            |
| <i>Chlorella</i> aff. <i>homosphaera</i> Skuja                                     |                 |           | x                |            |
| <i>Chlorococcum tatrense</i> P.A. Archibald  |                 |           | x                |            |
| <i>Chlorococcum microstigmatum</i> P.A. Archibald & Bold*                          | x               | x         |                  |            |
| <i>Chlorococcum</i> sp.  | x               | x         |                  |            |
| <i>Chlorococcal</i> sp. 3  |                 |           | x                |            |
| <i>Chloromonas alpina</i> Wille*   | x               | x         |                  |            |
| <i>Chloromonas fonticola</i> (R. Brabez) Gerloff & Ettl*                           | x               | x         |                  |            |
| <i>Chloromonas nivalis</i> (Chodat) Hoham & Mullet                                 | x               | x         |                  |            |
| <i>Chloromonas rostafinskii</i> (Starmach & Kawecka) Gerloff & Ettl*               | x               | x         |                  |            |
| <i>Chloromonas subdivisa</i> (Pascher & Jahoda) Gerloff & Ettl*                    | x               | x         |                  |            |
| <i>Dictyosphaerium</i> sp.   | x               | x         |                  |            |
| <i>Chlorocystidales</i>  |                 |           |                  |            |
| <i>Desmochloris halophila</i> (Guillard, Bold & McEntee) Watanabe, Kuroda & Maiwa* |                 | x         |                  |            |
| <i>Desmidiiales</i>  |                 |           |                  |            |
| <i>Actinotaenium curcubita</i> (Brebisson) Teiling                                 |                 |           | x                |            |
| <i>Mamiellales</i>   |                 |           |                  |            |
| <i>Ostreococcus</i> sp.  |                 | x         |                  |            |
| <i>Oedogoniales</i>  |                 |           |                  |            |
| <i>Oedogonium cardiacum</i> Wittrock ex Hirn*                                      |                 | x         |                  |            |
| <i>Prasiolales</i>   |                 |           |                  |            |
| <i>Desmococcus olivaceus</i> (Persoon ex Acharius) J.R. Laundon                    | x               | x         |                  |            |
| <i>Desmococcus spinocystis</i> Gärtner & Ingolic*                                  | x               | x         |                  |            |
| <i>Diplosphaera chodatii</i> Bialosukniá   | x               | x         |                  |            |
| <i>Diplosphaera</i> sp. 1  | x               | x         |                  |            |
| <i>Diplosphaera</i> sp. 2  | x               | x         |                  |            |
| <i>Elliptochloris marina</i> Letsch*   |                 | x         |                  |            |
| <i>Elliptochloris perforata</i> Hoffmann & Kostikov*                               |                 | x         |                  |            |
| <i>Elliptochloris reniformis</i> Darienko & Pröschold*                             | x               | x         |                  |            |
| <i>Koliella longiseta</i> (Vischer) Hindák*  | x               | x         |                  |            |
| <i>Koliella sempervirens</i> (Chodat) Hindák*                                      | x               |           |                  |            |
| <i>Prasiola</i> sp.  | x               | x         |                  |            |
| <i>Pseudochlorella signiensis</i> (Friedl & O'Kelly) Darienko & Pröschold          |                 | x         |                  |            |
| <i>Pseudochlorella subsphaerica</i> Reisiğl*                                       | x               | x         |                  |            |
| <i>Raphidonema nivale</i> Lagerheim  | x               | x         | x                | x          |

**Table 1** (continued)

| Taxa  | ASPA 140<br>HTS | WB<br>HTS | ASPA 140<br>Cult | WB<br>Cult |
|---|-----------------|-----------|------------------|------------|
| <i>Raphidonema pyrenoidifera</i> Korschikoff  |                 |           |                  | x          |
| <i>Stichococcus bacillaris</i> Wille  | x               | x         |                  |            |
| <i>Stichococcus mirabilis</i> Lagerheim   | x               | x         |                  |            |
| <i>Sphaeropleales</i>   |                 |           |                  |            |
| <i>Bracteacoccus bullatus</i> Fuciková, Flechtner & L.A. Lewis  | x               | x         |                  |            |
| <i>Chodatodesmus australis</i> Sciuto, Verleyen, Moro & La Rocca  | x               |           |                  |            |
| <i>Coenochloris signiensis</i> (Broady) Hindák  |                 |           | x                |            |
| <i>Desmodesmus</i> sp.  | x               | x         |                  |            |
| <i>Neocystis mucosa</i> Krienitz, C.Bock, Nozaki & M. Wolf*   | x               | x         |                  |            |
| <i>Neocystis</i> sp.  | x               | x         |                  |            |
| <i>Trebouxiales</i>   |                 |           |                  |            |
| <i>Asterochloris phycobiontica</i> E. Tschermak-Woess*  |                 |           | x                |            |
| <i>Chloroidium engadinensis</i> (Vischer) Darienko, Gustavs, Mudimu, Menendez, Schumann, Karsten, Friedl & Pröschold* | x               | x         |                  |            |
| <i>Coccomyxa subellipsoidea</i> E.Acton*  | x               | x         |                  |            |
| <i>Coccomyxa</i> sp. 1  | x               | x         |                  |            |
| <i>Coccomyxa</i> sp. 2  | x               | x         |                  |            |
| <i>Lobosphaera incisa</i> (Reisigl) Karsten, Friedl, Schumann, Hoyer & Lembcke*                                       | x               | x         |                  |            |
| <i>Myrmecia bisecta</i> Reisigl   | x               | x         |                  |            |
| <i>Trebouxia asymmetrica</i> Friedl & Gärtner*  | x               | x         |                  |            |
| <i>Trebouxia vaga</i> Voytsekhovich & Beck*   | x               |           |                  |            |
| <i>Trebouxia impressa</i> Ahmadjian*  | x               | x         |                  |            |
| <i>Trebouxia simplex</i> Tschermak-Woess*   | x               | x         |                  |            |
| <i>Trebouxia incrustata</i> Ahmadjian ex Gärtner*   | x               | x         |                  |            |
| <i>Trebouxia flava</i> P.A. Archibald*  | x               | x         |                  |            |
| <i>Ulotrichales</i>   |                 |           |                  |            |
| <i>Chlorothrix</i> sp.*   | x               | x         |                  |            |
| <i>Gloettilopsis planctonica</i> M.O.P. Iyengar & Philipose*  | x               | x         |                  |            |
| <i>Hazenia broadyi</i> Škaloud, Nedbalová, Elster & Komárek   | x               | x         |                  |            |
| <i>Planophila bipyrenoidosa</i> Reisigl*  | x               | x         |                  |            |
| <i>Planophila laetevirens</i> Gerneck*  |                 |           | x                |            |
| <i>Rhexinema</i> sp.  |                 |           | x                |            |
| <i>Ulvales</i>  |                 |           |                  |            |
| <i>Blidingia</i> sp.*   | x               | x         |                  |            |
| <i>Kornmannia leptoderma</i> (Kjellman) Bliding*  |                 |           | x                |            |
| <i>Pseudendoclonium commune</i> Darienko & Pröschold*   |                 |           | x                |            |
| <i>Pseudendoclonium submarinum</i> Wille*   | x               |           |                  |            |

HTS refers to identification by DNA metabarcoding in the present study. CULT refers to data reported by Fermani et al. [17] using culture techniques. \* indicates a taxon previously not recorded from Antarctica

obtained here, will have much larger distributions than currently known. It is also important to note the caveat that studies, such as the current, of eDNA found in soil do not generate voucher material or cultures of the taxa identified (Table 5).

Some of the taxa found are common symbionts with lichen-forming fungi, such as the genera *Asterochloris*,

*Diplosphaera*, and *Trebouxia*, and therefore, their geographical distributions are likely to be much wider. *Gloettilopsis planctonica* is a species of freshwater phytoplankton, whose presence in these soil samples is likely to be related to the close proximity of freshwater lakes to the sampling sites. The few marine species recorded (*Caulerpa webbiana*,

**Table 2** Abundance, distribution, and habitat of each taxon found at ASPA 140 subsite B (Crater Lake) and Whalers Bay (WB) in the current study

| Taxa                               | Geographical distribution | Habitat | Number of reads      |        |
|------------------------------------|---------------------------|---------|----------------------|--------|
|                                    |                           |         | ASPA 140 Crater Lake | WB     |
| <i>Asterochloris phycobiontica</i> | E                         | T       | 0                    | 14     |
| <i>Blidingia</i> sp.               |                           | M       | 175                  | 229    |
| <i>Bracteacoccus bullatus</i>      | A, E                      | T       | 957                  | 1393   |
| <i>Caulerpa webbiana</i>           | W                         | M       | 590                  | 2206   |
| <i>Chlamydomonas nivalis</i>       | W                         | S       | 911                  | 247    |
| <i>Chlamydomonas proboscigera</i>  | E, As                     | F/T     | 93                   | 1818   |
| <i>Chlamydomonas raudensis</i>     | E, A                      | F       | 0                    | 22     |
| <i>Chlorococcum microstigmatum</i> | E, NA                     | T       | 713                  | 2943   |
| <i>Chlorococcum</i> sp.            | E                         |         | 177                  | 2388   |
| <i>Chloroidium engadinensis</i>    | E                         | T       | 27                   | 44     |
| <i>Chloromonas alpine</i>          | E, As, Au, Nz             | F/T     | 50                   | 35     |
| <i>Chloromonas fonticola</i>       |                           | F       | 7150                 | 2714   |
| <i>Chloromonas nivalis</i>         | W                         | S       | 4724                 | 6201   |
| <i>Chloromonas rostafinskii</i>    | E                         | T/F     | 15,024               | 141    |
| <i>Chloromonas subdivisa</i>       | E                         | F       | 23,655               | 38,185 |
| <i>Chlorothrix</i> sp.             | E                         | M       | 28,329               | 41,351 |
| <i>Chodatodesmus australis</i>     | Ae                        | T/F     | 80                   | 0      |
| <i>Coenochloris signiensis</i>     | A, E                      | T       | 0                    | 12     |
| <i>Coccomyxa</i> sp. 1             | E                         |         | 1338                 | 206    |
| <i>Coccomyxa</i> sp. 2             |                           |         | 99                   | 10     |
| <i>Coccomyxa subellipsoidea</i>    | E                         | F       | 8                    | 33     |
| <i>Desmochloris halophile</i>      | NA                        | M       | 0                    | 58     |
| <i>Desmococcus olivaceus</i>       | A, Ar, W                  | A/T/C   | 1062                 | 239    |
| <i>Desmococcus spinocystis</i>     | E                         | T       | 4385                 | 12,198 |
| <i>Desmodesmus</i> sp.             |                           |         | 2715                 | 5241   |
| <i>Dictyosphaerium</i> sp.         | W                         |         | 40,624               | 53,382 |
| <i>Diplosphaera chodatii</i>       | W                         | T       | 3419                 | 2162   |
| <i>Diplosphaera</i> sp. 1          | W                         |         | 1042                 | 101    |
| <i>Diplosphaera</i> sp. 2          | W                         |         | 716                  | 84     |
| <i>Elliptochloris marina</i>       | NA                        | M       | 0                    | 62     |
| <i>Elliptochloris perforata</i>    | E                         | T       | 0                    | 43     |
| <i>Elliptochloris reniformis</i>   | A                         | T       | 47                   | 217    |
| <i>Gloeotilopsis planctonica</i>   | A, I, C                   | F       | 2671                 | 1479   |
| <i>Hazenia broadyi</i>             | Ae                        | F       | 775                  | 1228   |
| <i>Koliella longiseta</i>          | E                         | F       | 1439                 | 11,170 |
| <i>Koliella sempervirens</i>       | E                         | F       | 110                  | 0      |
| <i>Kornmannia leptoderma</i>       | W                         | M       | 0                    | 11     |
| <i>Lobosphaera incisa</i>          | E, NA                     | F       | 223                  | 291    |
| <i>Myrmecia bisecta</i>            | A, E, Au, NZ              | T       | 432                  | 296    |
| <i>Neochlorosarcina negevensis</i> | As                        | T       | 32                   | 33     |
| <i>Neocystis mucosa</i>            | A, E                      | F       | 489                  | 126    |
| <i>Neocystis</i> sp.               | E                         |         | 923                  | 670    |
| <i>Oedogonium cardiacum</i>        | W                         | F       | 0                    | 12     |
| <i>Ostreococcus</i> sp.            | E                         | M       | 0                    | 184    |
| <i>Planophila bipyrenoidosa</i>    | E                         | T       | 181                  | 201    |
| <i>Planophila laetevirens</i>      | E                         | T       | 0                    | 459    |
| <i>Prasiola</i> sp.                | W, A, Ar                  | F/T     | 3886                 | 3399   |

**Table 2** (continued)

| Taxa                                 | Geographical distribution | Habitat | Number of reads       |      |
|--------------------------------------|---------------------------|---------|-----------------------|------|
|                                      |                           |         | ASPAs 140 Crater Lake | WB   |
| <i>Pseudoclonium commune</i>         | E                         | M       | 0                     | 11   |
| <i>Pseudoclonium submarinum</i>      | W                         | M       | 294                   | 0    |
| <i>Pseudochlorella signiensis</i>    | A, E                      | T       | 0                     | 14   |
| <i>Pseudochlorella subsphaerica</i>  | E                         | T       | 86                    | 11   |
| <i>Raphidonema nivale</i>            | A, E, SA, As, Au, NZ      | F       | 85                    | 85   |
| <i>Rhexinema</i> sp.                 |                           |         | 0                     | 1053 |
| <i>Sanguina nivaloides</i>           | W                         | F/S     | 0                     | 2075 |
| <i>Stichococcus bacillaris</i>       | Ar, E, SA                 | U       | 806                   | 1482 |
| <i>Stichococcus mirabilis</i>        | Ar, E, SA                 | F       | 2254                  | 6499 |
| <i>Tetracystis diplobionticoidea</i> | NA                        | T       | 0                     | 393  |
| <i>Tetracystis</i> sp.               |                           |         | 153                   | 0    |
| <i>Tetracystis vinatzeri</i>         | E                         | T       | 1537                  | 853  |
| <i>Trebouxia asymmetrica</i>         | E                         | T       | 2101                  | 1529 |
| <i>Trebouxia flava</i>               | E                         | E       | 263                   | 46   |
| <i>Trebouxia impressa</i>            | E, As                     | T       | 156                   | 7    |
| <i>Trebouxia incrustata</i>          | E                         | T       | 345                   | 8    |
| <i>Trebouxia simplex</i>             | E                         | T       | 4019                  | 3389 |
| <i>Trebouxia vaga</i>                | E                         | T       | 333                   | 528  |

Geo geographic distribution: A Antarctica, Ae Antarctic endemic, Ar Arctic, Au Australia, As Asia, E Europe, C China, I India, NZ New Zealand, SA South America, W cosmopolitan. Habitat: A aerial, C chasmoendolithic, F freshwater, M marine, NA North America, S snow, T terrestrial, U ubiquitous. Taxa at genus level may be missing habitat or geographical distribution data

**Table 3** Ecological indices of green algal assemblages from ASPA 140 subsite B and Whalers Bay in the current study and from Mt. Erebus [30] and Edward VII Peninsula [43]

| Indices         | ASPAs 140 (Crater Lake) | Whalers Bay | Mt. Erebus [30] | Edward VII Peninsula [43] |
|-----------------|-------------------------|-------------|-----------------|---------------------------|
| OTU number      | 50                      | 61          | 14              | 9                         |
| Fisher $\alpha$ | 3.52                    | 5.79        | 4.55            | 4.07                      |
| Margalef        | 3.06                    | 4.87        | 2.81            | 2.28                      |
| Simpson         | 0.85                    | 0.86        | 0.90            | 0.81                      |

OTU operational taxonomic unit. Broady papers did not assess OTUs, and the species numbers given were assessed by direct morphological assessment

*Desmochloris halophile*, *Elliptochloris marina*, *Kormmannia leptoderma*, *Pseudoclonium commune*, *P. submarinum* were found mostly in Whalers Bay, except *P. submarinum* which was present only in ASPA 140, which may be explained by the much closer proximity to the coast in the former location. Several of these species currently have very

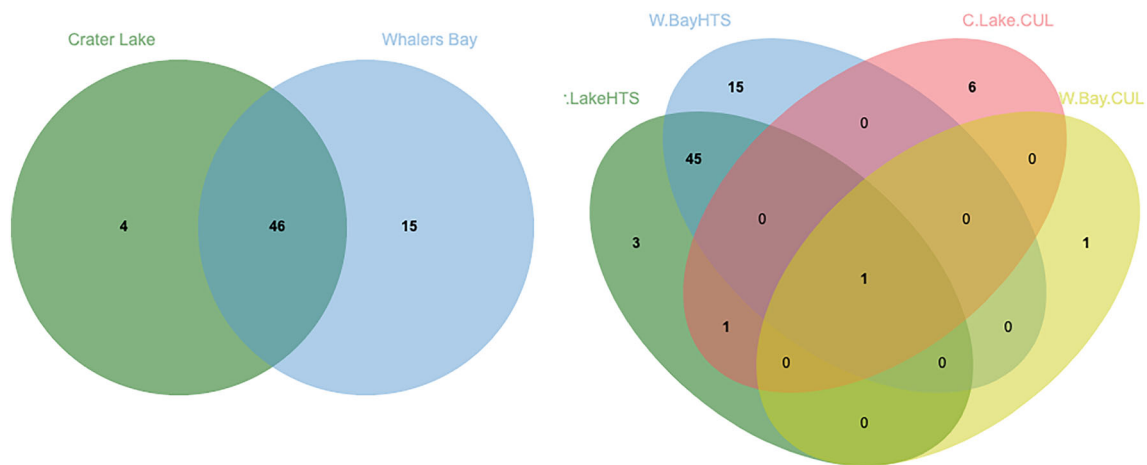
disjointed distributions globally: *Pseudoclonium commune* is a photobiont found on coastal rocks in the North and Irish Seas associated with *Pelvetia canaliculata* (Phaeophyceae/Fucales) beds [52]; *E. marina* lives in symbiosis with sea anemones of the temperate Pacific Ocean in the Northern Hemisphere; *D. halophile* is an epibiont of a marine

**Table 4** Ecological indices of green algal assemblages (at genus level) from ASPA 140 subsite B and Whalers Bay in the current study and extracted from Garrido-Benavent et al. [27]

| Indices                            | ASPAs 140 (Crater Lake) | Whalers Bay | <i>p</i> value diversity <i>t</i> test | Garrido-Benavent et al. [27] |
|------------------------------------|-------------------------|-------------|--|------------------------------|
| Shannon                            | 2.45                    | 2.56        | $1.65e^{-137}$                         | 1.8–2.5                      |
| Simpson_1-D                        | 0.85                    | 0.86        | $2.75e^{-119}$                         | 0.75–0.85                    |
| Equitability_J (Pielou's evenness) | 0.59                    | 0.65        |  | 0.5–0.68                     |

OTU operational taxonomic unit





**Fig. 2** Venn diagram showing Chlorophyta taxa distribution and shared species between both study sites. Left, HTS data alone; right, HTS data plus culture data from Fermani et al. [17]

species of *Cladophora* in Wisconsin; and *K. leptoderma* is widely distributed in the Arctic, North Atlantic, Alaska, and Asia.

Pellizzari et al. [53] identified the seaweed *Monostroma grevillei* on Deception Island. This species has a biogeographic distribution similar to *K. leptoderma*, and both are considered cryptic monostromatic chlorophytes. Pellizzari et al. [53] suggested that *M. grevillei* may have been introduced to Deception Island during whaling activity early in the twentieth century, in which ships of North Atlantic origin played a large role. *K. leptoderma* might represent a similar case, demanding further and detailed investigation. Most of the marine taxa present were recorded with very low abundance. However, *C. webbiana* displayed a higher abundance (2206 reads), which is potentially of great concern as this taxon is one of the most invasive macroalgae [54, 55]. Although currently considered to be restricted to warmer waters, it has been reported as far south as New Zealand (Norfolk Island), and the volcanic activity in Deception Island, causing water heating within Port Foster, may provide suitable conditions for the establishment of this organism that has, so far, not been reported in Antarctic waters [53].

Members of the genus *Desmodesmus* have been reported as potentially causing human infections [56]. The taxon was

present at both current study sites, but with higher abundance in Whalers Bay. The widespread *Stichococcus mirabilis* was also very abundant especially in Whalers Bay and has been reported in South America as close as Argentina [57]. Taxa such as *Chlorella* and *Stichococcus* may be capable of inter-continental dispersal [57], as may also be the case for many Chlorophyta due to their small size and high resistance towards environmental stresses. Deception Island hosts a diversity of bird species, with species such as *Larus dominicanus*, *Catharacta antarctica*, and *Chionis alba* migrating between and common within the maritime Antarctic, southern South America, and the sub-Antarctic islands.

Human influence can also not be ruled out as a possible agent dispersing green algae to Antarctica. Many of the taxa reported here are also known from European locations, the origin of many tourist and national operator visitors to Deception Island. As a relatively young volcanic island still undergoing colonization, Deception Island is an important natural laboratory in which to study the taxonomy, ecology, and evolution of both resident and non-native species under extreme conditions.

In conclusion, our results confirm that DNA metabarcoding was able to unveil a hidden algal community present in the soils of Deception Island, identifying a much higher level of diversity than previously recorded using traditional culture methods. In addition, although only two locations were sampled, there appeared to be important differences between the protected and the strongly human-impacted site in terms of diversity, richness, and abundance. As the western Antarctic Peninsula region is among the parts of the planet most affected by recent climatic changes, the detection of DNA of taxa from multiple different parts of the world highlights concerns about the potential impact of tourism and/or scientific activities on the future biological colonization of Antarctica.

**Table 5** Compositional dissimilarities among assemblages.

| Beta diversity indices | ASPA 140 (Crater Lake) | Whalers Bay |
|------------------------|------------------------|-------------|
| $\beta_{sor}$          | 0.94                   | 0.94        |
| $\beta_{sim}$          | 0.59                   | 0.75        |
| $\beta_{sne}$          | 0.34                   | 0.18        |

$\beta_{sor}$ , Sørensen index of compositional dissimilarity;  $\beta_{sim}$  turnover component of compositional dissimilarity;  $\beta_{sne}$ , nestedness component of compositional dissimilarity

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**Authors’ Contribution** PEASC collected soil samples, optimized protocols for DNA extraction, and wrote the first version of manuscript. MCS contributed significantly in the later versions of manuscript. OHBP filtered the data and performed the metagenomic analysis once DNA information was available. ETA performed the ecological analyses. DKH helped optimizing laboratory protocols and revised all the manuscript versions. THS worked with protocol optimization and gave inputs to methodology. FP provided significant contributions to the manuscript discussion and revised all its versions. PC contributed to the result interpretations, gave important feedback to discussion and revised all manuscript versions. LHR collected the soil samples, provided the necessary infrastructure for DNA extraction, helped writing the first version of manuscript, and revised all versions.

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**Data Availability** All soil samples analyzed in this paper are stored in the Laboratory of Microbiology at Universidade Federal de Minas Gerais.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethics Approval** The collections and studies performed in ASPA 140 were authorized by the Secretariat of the Antarctic Treaty and by PROANTAR.

**Code Availability** Not applicable.

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