# METHODS

## Site description

Location

Scott Base is located Pram Point, in Hut Point Peninsula, Ross Island, Southern Victoria Land, Antarctica (Fig. A) at approximately -77.848797, 166.767922. The measuring probes were located in the base of a hill, slightly north to the base (Fig. A). We got the climatic data from the Scott Base Climatic Station which is situated uphill from the Base, on a side slope at 38 m elevation (Seybold et al., 2009).

A screenshot of a satellite image

Description automatically generated

*Figure A.*

Geology, topography and climatic conditions

Pram Point is located in the McMurdo Volcanic Formation (Sheppard et al., 2000). The soil is mainly formed by flows of strongly unsaturated alkaline basal (Kyle, 1981; Sheppard et al., 2000), and consists of loosely compacted stony gravelly sand (Council of Managers of National Antarctic Programs, 2017). The tectonic history of the area remains unclear (Kyle, 1981).

The base is located at 10 m of altitude, at the side of a hill that gently slopes southwards towards the sea (Council of Managers of National Antarctic Programs, 2017), and ice has modified the surface (Sheppard et al., 2000). Permafrost occurs at 30 cm depth (Council of Managers of National Antarctic Programs, 2017).

This topography diverts air from the south, so at the Base the main winds blow from the north-east, whereas at higher altitudes they predominantly come from the south and are stronger (Sheppard et al., 2000). The mean wind speed is 19.1 km h-1 (Council of Managers of National Antarctic Programs, 2017). Water content of the soil over the permafrost is around 6% (Seybold et al., 2009), and average mean soil temperature is one degree higher than the air temperature that hovers around -19.4 °C (Seybold et al., 2009). This is because during the summer the soil gets warmer than the air due to the constant solar radiation 24 h a day (Seybold et al., 2009). There is no records of precipitation patterns, but there is snow all year round (Council of Managers of National Antarctic Programs, 2017).

Management

The area hosts New Zealand’s main Antarctic research base, and it has an occupation of 10 people during the winter season and around a 100 during the summer (Sheppard et al., 2000). The United States’ McMurdo Station is also located in Hut Point Peninsula, and it hosts more that 1000 people during the summer (Lohrer et al., 2023). Both stations serve as research bases and posts for expeditions further inland(Sheppard et al., 2000). As a result of the human activity in the area, the environment around the base has been highly modified, resulting in a reduction in moss, lichen, and snow cover (Sheppard et al., 2000). Spills of waste, oils and chemicals have polluted the soils closer to the base (Aislabie et al., 2000; Lohrer et al., 2023; Sheppard et al., 2000).

Biodiversity

There are diverse biological communities of organisms in the area, including mosses, lichens, algae and soil invertebrates(Council of Managers of National Antarctic Programs, 2017). Lichen’s biodiversity is higher than mosses’ in a ratio 30:7(Green et al., 2007). They are sparsely distributed in small patches, with higher abundance in moist low-disturbed areas (Council of Managers of National Antarctic Programs, 2017; Seppelt & Green, 1998). Weddell seals (*Leptonychotes weddellii)* and South polar skua (*Catharacta maccormicki*) can be abundantly found in the mid-summer (Council of Managers of National Antarctic Programs, 2017).

## Investigated Species

The species studied are *Bryum argenteum* (Fig. BA) and A*ustroplaca soropelta* (Fig. BB). *Bryum argenteum* was previously referred to as *Bryum subrotundifolium* when refereeing to the variety *muticum*, but this is no longer valid (Gemal et al., 2022; Ochyra et al., 2008). *Austroplaca soropelta* was previously refer to in the literature as *Caloplaca soropelta* (Garrido-Benavent & Pérez-Ortega, 2017).

*Bryum argenteum* is a cosmopolitan moss with a strong presence on the Antarctic (Gemal et al., 2022)– Bryum is the most widespread genus of moss across continental Antarctica (Pannewitz et al., 2005; Seppelt & Green, 1998), and the species *argenteum* the most widespread species across Victoria Land (Gemal et al., 2022). It grows in disturbed soils in moist or wet sites with soft and rocky ground (Pannewitz et al., 2005; Schlensog et al., 2004). Its wide distribution has resulted in it being extensively studied, but it has also created a lot of taxonomic confusion (Gemal et al., 2022; Seppelt & Green, 1998). The appearance of *Bryum argemteum* changes between sun and shade adapted organisms. Whereas the sun variety is more yellowish and densely packed, the shade form is darker and less densely packed. Both forms blend into one another, with the shade one being less common (Schroeter et al., 2012). For a full morphological description refer to Seppelt & Green (1998).

*Austroplaca soropelta* is mainly spread in the Southern Hemisphere (Garrido-Benavent & Pérez-Ortega, 2017) – the genus *Austroplaca* is one on the largest genera is Antarctica (Søchting & Castello, 2012) – but it is also found in northern latitudes like Iceland or Greenland(Søchting & Castello, 2012). It grows in strongly convex moss cushions, and it is characterized by initially convex yellow lobes (Søchting & Castello, 2012). For a full morphological description refer to Søchting & Castello (2012).

## A close-up of a device Description automatically generated

*Figure B.*

## Data collection

A Pulse Amplitude Modulation fluorometer (MoniDa) was installed (Fig. B) (Raggio et al., 2014, 2016; Schroeter et al., 2011). Each of the 4 probes monitor a sample – 1,2 and 4 correspond to *Bryum argenteum* and probe 3 measures the activity of *Austroplaca soropelta*. Each probe is equipped with temperature and light sensors that measure thallus temperature (TT) and photosynthetic active radiation (PAR), and one fiber optic that monitors the physiological performance of the sample (Raggio et al., 2016).

Measurements are taken every hour, when the sample is illuminated with a low intensity modulated light to record fluorescence (*Ft*) followed by a saturating flash of actinic light. After the flash the resulting maximal fluorescence (*Fm*) is recorded and the effective quantum use efficiency of PSII, or Yield, can be calculated . Relative Electron Transport Rate (ETR) of the PSII is also calculated ) and can be used as a proxy for photosynthetic CO2 fixation (Raggio et al., 2016). For a full description of the methodology refer to Raggio et al. (2014), and for an in-depth explanation of the use of florescence to measure photosynthetic activity refer to Baker (2008), Kromdijk and Walter (2023), Johnson (1993), and Maxwell & Johnson (2000).

Measurements started on January 18th, 2019, and continue to present (March 2024). I used measurements from January 18th, 2019, until 1st of November 2023. Time and date of each of the measurement were also recorded.

Air temperature, and relative humidity (RH) were collected from the Scott Base Climatic Station. Every hour average, minimum and maximum values for each of the variables were recorded.

## Data Processing

Screening of the data

Lower estimates of Fm and Ft can give deceivingly high Yield values because of the proportional nature of the latter (Maxwell & Johnson, 2000). MoniDA has an internal data filter to avoid this from happening, so the machine will not record any yield values () (Raggio et al., 2016) if Ft is below 10 measuring units of Fm below 50. However, in some cases, this can give a false sense of inactivity and hide trends when significant differences between Ft and Fm exists and the fluorometer’s shut down. To avoid missing important information, I manually calculated the Yield and ETR of each measurement and compared this value to the machine’s recorded value in each activity period. I filtered measurements that showcased a discrepancy between both values. If they had a significant *Fm-Ft* difference (Fm-Ft > 5) (T. Green, personal communication, 11th December 2023) I used the calculated yield, and if Fm-Ft was not significant I maintained the machine’s measurement of inactivity.

Definition of activity and activity periods

We consider as activity every value of Yield after the screening (Raggio et al., 2014). This is a measure of photosynthetic performance instead of metabolic activity (Raggio et al., 2016), but there were no events in which we found metabolic activity () and no yield.

I plotted the data for the whole measuring period to assess the behavior under snow and microclimatic conditions of all samples throughout the whole year. However, I limited statistical comparisons and in-depth graphical observations to the “active periods”. After observing the activation patterns for the whole measuring period and comparing it to measuring times in previous literature, I considered *active periods* in the context of this paper, as time between the 1st of November and last day of February each year (Colesie et al., 2016; Gemal et al., 2022; Pannewitz et al., 2003; Schlensog et al., 2004, 2013). This definition covers the entirety of the Antarctic summer and includes the snowmelt at the beginning of the season and the snowfall at the end, allowing observations of activity under snow for both species.

I used R Studio (Posit team, 2024) and the Tidyverse package (Wickham et al., 2019) to wrangle and format all the data.

## Data analysis

There were three replicas of *Bryum argemteun* and one replica or *Austroplaca soropelta*. Since I did not have a statistically relevant number of samples, I could not conduct any parametrical statistics. Therefore, most of this study was observational and used descriptive statistics. I used non-parametrical statistics after observing a decline in active time over the years, to test the significance of it and the correlation to possible drivers.

Observational Study: climatic conditions in Scott Base, characterization of activity and activation patterns and behaviour under snow.

Yield, TT, Air temperature, RH and PAR were plotted against time for the whole measuring period, and each summer (Fig. 1 and 3). And used to assess patterns over time and responses to changes in microclimatic conditions.

Between the 15th and 19th of March 2023 a heat wave spread over East Antarctica with temperature anomalies of 30 to 40 °C (Wille et al., 2024). At the peak of the heatwave, an area of 3.3 million km2 including Victoria Land,exceeded monthly temperatures expected during March (Wille et al., 2024). I plotted a closeup during this period to observe for any anomalies on microclimatic or photosynthetic performance in either of the species.

Snowmelt and snowfall events were identified in the graphs as moments when TT was ~0 °C and there was a no PAR available (Pannewitz et al., 2003). I generated closeup activity and microclimate graphs for each of them to assess and compare the behaviour of both species under the snow.

I also calculated descriptive statistics for TT, yield, ETR, PAR, and in the case of the meteorological data air temperature and RH. Mean, maximum and minimum values were extracted for each sample and species across the whole period, as well as each month and summer.

Statistical analysis: decline of active time over the years and possible correlations.

I tested whether there was a significant difference in the number of active hours per year using a Kruskal Wallis test. This test allows to measure the difference between groups even if they do not meet the assumptions of normality and independency(McKight & Najab, 2010; Vargha & Delaney, 1998).

I then assessed whether there was a correlation between i) the number of active hours and TT of the sample during active time, and ii) number of active hours and the number of reactivation events according to two metrics:

1. *High* metric: A sample had a reactivation event when there was at least one measurement (one hour) in which followed by at least one measurement (1 hour) in which .
2. *Low* metric: A sample had a reactivation event when there was at least one day (24 measurements) in which followed by at least one day (24 measurements) in which

I used the Spearman’s correlation test to measure these and backed it up with a Kendal Tau test to increase reliability. These two tests are the most widely accepted measures of rank correlation and are appropriate when extreme values are present (Mukaka, 2012; Puka Llukan, 2011). I only considered as significant those results in which both tests gave similar values for the correlation coefficient and its significance. Since all the samples showed similar changes in TT and activation patterns (Fig. 1), I analysed measurements for all samples together independently of the species. This increased sample size, increasing the reliability of the tests. Limitations of these methodology are discussed further down.

I also plotted these correlations grouping by sample and species to observe whether there were any clear tendencies (Appendix 4).

I used R Studio (Posit team, 2024) and the Tidyverse package (Wickham et al., 2019) to create and the graphs and statistical analysis.

All the graphs and code are available on GitHub: <https://github.com/Ainhoa-Jimenez/dissertation.git>

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