Chapter 5

Leaching of glyphosate and AMPA in two sub-Antarctic soils and potential ecosystem impacts of glyphosate application on Macquarie Island

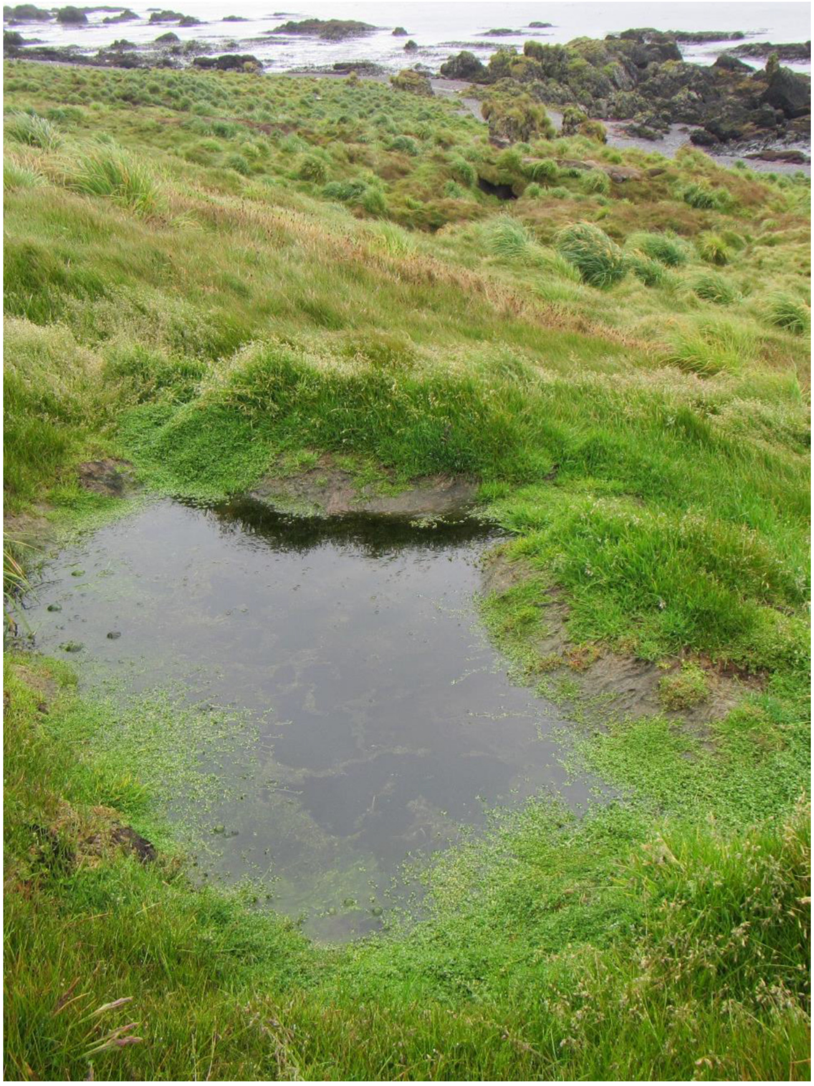

Plate 5 *Poa annua* growing in a wet environment on Macquarie Island

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This chapter has been removed as it awaiting publishing.

Chapter 6

General discussion

Plate 6 Lake on the plateau of Macquarie Island

General discussion

Invasive species pose major threats to the sub-Antarctic and Antarctic (Frenot et al. 2005; Chown et al. 2012). In the broader Southern Ocean Islands group, eradication of small populations of non-native plants have been successful, and control of larger populations is being attempted (Hughes et al. 2015; McGeoch et al. 2015). Control of the most widespread weed in the sub-Antarctic and Antarctica, *Poa annua,* is being attempted in the Antarctic Peninsula (Chwedorzewska unpublished data) and feasibility of its eradication from sub-Antarctic Heard Island is being assessed (de Villiers et al. 2006; Commonwealth of Australia 2014). To date, eradication of widespread species has been largely unsuccessful, mostly due to established seed banks (Cooper et al. 2011; Ryan et al. 2012; Shaw 2013). Information on the biology of the target species and efficacy and impact of management are often missing from management programs but are required for successful eradication or control. This has previously been lacking for the most widespread weed in the sub-Antarctic, *P. annua*.

This thesis provides essential information on the ecology of *P. annua* on sub-Antarctic Macquarie Island, including importantly, its seed bank dynamics. For a widespread species such as *P. annua,* which has established seed banks, knowledge of the size of the seeds banks and viability and persistence of seed is essential to determine how intensive management needs to be and how long is required to deplete the seed bank. It also quantifies for the first time, the efficacy and broader impact of various control methods, including the application of the widely used herbicide glyphosate, on an invasive plant, *P. annua*, in the sub-Antarctic environment. This information is critical for informing any future management of *P. annua.* Although the biology and ecology of the highly plastic *P. annua* has been well studied in temperate turf grass (i.e. Youngner 1959; Beard 1978; 1996; Warwick 1979, Hutchinson & Seymour 1982; Vargas & Turgeon 2004), little is known about the life history and ecology of populations specific to the sub-Antarctic. Previous observations suggested *P. annua* was perennial in the sub-Antarctic, however, through monitoring, I have quantified that *P. annua* populations on Macquarie Island are perennial. Additionally, I found that even within the relatively small island area of Macquarie Island, plants are highly plastic in morphology and in growth across environmental gradients such as elevation, animal disturbance and soil properties. *Poa annua* plants are large at low altitude, coastal sites where *P. annua* cover is high and there is much animal disturbance and deep, sandy soils. Conversely, at high altitude, exposed sites with no animal disturbance and shallow, gravely soils, *P. annua* plants are small. Although variable in size, *P. annua* plants on Macquarie Island allocated most of their biomass to vegetative growth, rather than reproduction, allowing them to persist in the harsh climatic conditions. However despite the relatively low investment in sexual reproduction, their reproductive output was still sufficient to enable population persistence. In addition, *P. annua* suppressed native plant diversity when growing at high densities.

Persistent seed banks are a particular problem affecting success of plant eradications of widespread invasive plants (Vranjic et al. 2000; Grundy & Jones 2002; Panetta & Timmins 2004; Gioria & Osborne 2010). I found that the seed bank density of *P. annua* varied greatly on Macquarie Island in response to environmental gradients. There were very high seed densities at low elevation coastal sites, with high wildlife disturbance and conditions such as low exposure and deep, sandy soils that enhanced growth and reproduction of *P. annua* and assisted in seed bank formation. Conversely, at high elevations, seed banks were small or non-existent because the high exposure and shallow, gravely soils reduced plant development and reproductive capacity, thus hindering seed bank formation. In seed burial trials, only 3 % of the seed remained viable after two years, however given the high potential seed bank densities at low altitude this means substantial number of seeds will still persist.

Many plant eradication programs use a trial-and-error approach, developing the best methods as the program progresses (Rippey et al. 2002; Milne 2007; Hilton & Konlechner 2010; Cooper et al. 2011; Lombard et al. 2012; Ryan et al. 2012; Hamilton et al. 2015). This approach can be slow, inefficient, ineffective and costly. Programs with prior knowledge on efficacy of control methods are much more successful. This is particularly important for a species such as *P. annua* where successful control is dependent on a number of factors including climatic conditions, specific *P. annua* populations, and non-target species. Therefore, conditions on Macquarie Island such as vigorous growth of *P. annua,* presence of native plant species and the cold, wet, windy environment may affect the efficacy of control methods and off-target impacts, particularly of herbicides.

I found that on Macquarie Island, physical control methods, such as scalping, hoeing, trimming and hand weeding, when applied only once were largely ineffective on *P. annua* and had some (but not significant) negative impact on native species richness. Physical control in the form of hand weeding, however, may be useful when used with other methods such as herbicides in an integrated weed management program. Several herbicides (glyphosate, rimsulfuron and trifloxysulfuron sodium) were effective at killing *P. annua* plants, even at sub-Antarctic temperatures. However the only herbicide treatment which was selective between *P. annua* and three common native grass species was glyphosate at 0.25 times the recommended label rate. Both brush and spray application methods were equally phytotoxic to *P. annua*. An integrated weed management program may be effective for the control of *P. annua* on Macquarie Island, with glyphosate used to initially kill above ground biomass and prevent further seed production in dense infestations, and subsequent spot-spraying of glyphosate and hand weeding of emerging seedlings and control of scattered plants. I have shown that seeds can persist in the soil for at least two years, so control of emerging seedlings will be required for several seasons following the control of above ground plant material to prevent seed set and ensure the seed bank is depleted.

While I have found that low rates of glyphosate can give effective selective control of *P. annua* on Macquarie Island, nothing is known about its fate and behaviour within the ecosystem. *Ex-situ* column studies suggested glyphosate does leach in sub-Antarctic Macquarie Island soils, at mean concentrations of 2.5 $\mu g\ L^{-1}$ and 5 $\mu g\ L^{-1}$ in sand and peat respectively. Some degradation to aminomethylphosphonic acid (AMPA), which can be more toxic than glyphosate, does also occur. However over the 48 week study period, only around 0.4 % of the initial glyphosate applied at a recommended field rate leached, with the rest remaining sorbed to the soil. Leachate concentrations were significantly lower than guideline values and values known to have ecotoxicological effects in other studies. I have also shown that rates as low as 0.25 times the recommended rate can kill *P. annua,* so at these application rates concentrations of glyphosate and AMPA in the environment would be further reduced. Despite this, commercial formulations can be more toxic (Folmar et al. 1979; Cox 1989; Duke & Powles 2008), repeat applications may expose biota to higher concentrations, and there is greater potential sensitivity of biota at colder temperatures (Helander *et al.* 2012). Therefore further long term *in situ* research is required on glyphosate toxicity to native flora and fauna before application is integrated into control programs.

Implications for the management of *Poa annua* in the sub-Antarctic

This thesis has addressed the three major shortcomings of control and eradication programs outlined in the introduction. Firstly, I have increased the understanding of the ecology of *P. annua* on Macquarie Island which has implications for management:

- *Poa annua* on Macquarie Island is perennial, which enables it to persist in the sub-Antarctic environment. Therefore control efforts will need to be on-going rather than once off and involve removal of existing plants to prevent seedling establishment.
- *Poa annua* is highly variable in its morphology and growth in the sub-Antarctic, even across small spatial scales, and therefore different populations will require different management techniques or approaches. For example, herbicides may be the quickest, most effective means of controlling dense infestations of *P. annua* and where there is little native species plant cover to be potentially affected. Conversely, spot spraying or hand weeding of scattered plants in areas of high native vegetation cover would minimise impacts to native plants.
- Seed banks are highly variable. In dense infestations of *P. annua* intensive active management would be required for at least two years to prevent further seed set and deplete the soil seed bank. Conversely, in areas with low *P. annua* cover and low seed bank density, prevention of further seed set and control of emerging seedlings will be quicker, easier and more effective.
- Seed banks are mostly concentrated within the top few centimetres of the soil implying management can also be restricted to the top of the soil profile, where the seed bank can be more easily and quickly depleted.
- The seed bank viability of most *P. annua* seed is very low (< 2 years). Therefore above ground biomass of *P. annua* can be controlled and further seedling establishment prevented, as most of the seed bank will be depleted within several years. However, in areas with dense seed banks (even at 3 % viability) considerable amounts of seed may persist longer than two years and so active management will be required to prevent seedling establishment.

Secondly, I have assessed the efficacy of a number of non-chemical and chemical control methods and determined which methods are appropriate for *P. annua* control in the sub-Antarctic:

- Physical methods used in isolation are not effective for the control of *P. annua* on Macquarie Island, particularly on dense infestations where it is difficult to remove plants from the soil. However, they may be beneficial in an integrated weed management program. Glyphosate at low rates appears to be effective and selective on *P. annua* and therefore could be used to control *P. annua* and prevent further seed production. Hand weeding or spot spraying could then be used to target emerging seedlings.
- Where *P. annua* has low cover at high altitudes, these areas should be targeted first for control, as control is likely to be more effective here due to low seed bank densities and low *P. annua* cover.

Thirdly, I have assessed the impact of non-chemical and chemical control methods on off-target native grasses and potential impacts of glyphosate use on Macquarie Island:

- Physical control methods caused some changes to native species richness and diversity but as they were not effective on *P. annua* anyway this is not of concern.
- Although rimsulfuron and trifloxysulfuron sodium were effective on *P. annua,* they were not selective of native grass species and so are not appropriate for use on Macquarie Island
- While glyphosate shows potential for *P. annua* control, low concentrations of glyphosate and its breakdown product AMPA will leach at low concentrations in a sand and peat soil from Macquarie Island. Although the concentrations of these compounds are well below levels likely to affect native biota, further *in situ* toxicity assessments are required.

Recommendations for future work

Prior to control of widespread *P. annua* in the sub-Antarctic, further research is required.

- Additional information on the life history of the species (e.g. time taken to reach reproduction) will determine frequency of post-control monitoring of emergent seedlings

- Hand weeding should be trialled in targeted areas with low infestations of *P. annua* on Macquarie Island to establish its effectiveness as part of an integrated control program and to see if the seed bank can be readily exhausted in the field.
- Regulatory constraints prevented the application of herbicides on Macquarie Island. Herbicides may act differently in the field to *ex situ* and so should be trialled on a small-scale on Macquarie Island to determine their field efficacy and impact.
- Likewise, although *ex-situ* column studies provide evidence that glyphosate may exhibit some leaching and persistence in Macquarie Island soils, they are not well representative of field conditions (rainfall, water flow etc.) and therefore leaching and persistence needs to be studied under field conditions, in several different soils. Additionally, the glyphosate anion was used in this study as it was easier to identify in complex chemical analyses, however, commercial formulations of glyphosate contain a number of different substances which may influence herbicide behaviour and toxicity and so should be incorporated into further study.
- While low rates of glyphosate show potential for *P. annua* control and appear selective of native grasses, further assessment is needed under field conditions as repeat applications may be more toxic, and Macquarie Island biota, particularly aquatic organisms, may be more sensitive. Toxicity tests need to be undertaken using commercial formulations of glyphosate as additional compounds such as surfactants may increase glyphosate toxicity.

Conclusions

This thesis has filled a number of gaps in knowledge of the ecology of *P. annua* in the sub-Antarctic, critical information to increase understanding of how this highly variable species behaves in the sub-Antarctic and why it is such a successful invader. I have also determined which control methods can be used to effectively control *P. annua* in the sub-Antarctic without causing off-target impacts. This information will be critical in informing any potential control or eradication programs for *P. annua* not only across the sub-Antarctic, but potentially in any cold-climate region.

On a broader scale, I have expanded knowledge of the traits that enable invasive species to persist in the sub-Antarctic environment and have determined what impact control methods can have on native species and environments in the sub-Antarctic. I have also emphasized the importance of capturing information on the ecology and efficacy and impact of control methods prior to management, to ensure programs are efficient and effective. The importance of this is highlighted by a recent incursion on Macquarie Island. In 2014 two previously unrecorded non-native grasses, *Agrostis stolonifera* and *Agrostis capillaris* were detected and subsequently removed by digging out plants, roots and soil (Pertierra *et al.* 2016). However, this has been unsuccessful and both species have reappeared (Department of Primary Industries, Parks, Water and Environment, unpublished data). *Agrostis stolonifera* in particular is highly invasive in the sub-Antarctic and can spread it high rates (le Roux *et al.* 2013). By having information on the impact of control methods in the sub-Antarctic, particularly herbicide use, we can hit the ground running and quickly control new incursions without the risk of unexpected impacts.

Chapter 7

References

Plate 7 Snow on the plateau of Macquarie Island

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Appendices

Appendix 1 – Publication arising from chapter 2

[redacted]

Appendix 1 has been removed as it was published as the following journal article, arising from Chapter 2 of this thesis:

Williams, L., Kristiansen, P., Shaw, J., Sindel, B., & Wilson, S.C. (2013) Weeds down under: Invasion of the sub-Antarctic wilderness of Macquarie Island. *Plant Protection Quarterly*, 28 (3), 71-72.

Appendix 2 Paper arising from Chapter 4

[redacted]

Appendix 2 has been removed as it was published as the following journal article, arising from Chapter 4 of this thesis:

Williams, L., Kristiansen, P., Sindel, B., Wilson, S., & Shaw, J. (2016). Quantifying the seed bank of an invasive grass in the sub-Antarctic: seed density, depth, persistence and viability. *Biological Invasions*, 18(7), 2093-2106. http://dx.doi.org/10.1007/s10530-016-1154-x

Appendix 3 Assessment of the efficacy of various methods for the control of *Poa annua* presented in the literature

Control method	Efficacy	Reference
HERBICIDES		
Amicarbazone	Some control (timing and temperature dependent)	McCullough & Hart (2010a)
	Effective control	Jeffries *et al.* (2013)
Amitrole	Some control	Finlyson *et al.* (2000)
Atrazine	Reduced growth but didn't eradicate	Lee (1981)
Benefin	Effective control (in *Cynodon dactylon* turf but non-selective)	Downing *et al.* (1970)
	Effective control	Bingham *et al*. (1979)
Bensulide	Some control (independent of biotype)	Juska *et al.* (1967); Warwick *et al* (1980)
	Reduced growth (*C. dactylon* turf but non-selective)	Downing *et al.* (1970)
	Effective control (in *Agrostis tenuis* turf)	Jagschitz (1970)
	Variable control	Bingham *et al.* (1979)
	Effective control (in *Agrostis stolonifera* turf but non selective)	Breuninger (1993); Teuton *et al.* (2007)
Bispyribac-sodium	Effective control (in *Agrostis palustris* turf)	Park *et al.* (2002)
	Effective control (in *C. dactylon* turf)	McElroy *et al.* (2004)
	Effective control (post-emergent in turf)	Branham & Calhoun (2005)
	Some control (in *A. stolonifera* and *Lolium perenne* turf - dependent on additives)	Lycan *et al*. (2006); McCullough & Hart (2006); McCullough *et al.* (2009a; 2010b)
	Effective control (in *A. stolonifera* turf but temperature, timing dependent)	McCarty & Estes (2005); McCullough *et al.* (2009a; 2011)
Butralin	Effective control	Bingham *et al.* (1979)
Calcium arsenate	Effective control (but phosphorus dependent)	Juska *et al.* (1967)
Carbetamide	Effective control	Xiao *et al.* (2006)
Chloropropham	Reduced growth but not eradication	Lee (1981)
Chlorosulfuron	Effective control (in *A. stolonifera* and *Poa pratensis* turf, also selective)	Gaul *et al.* (1987)
Chlorthal-dimethyl	Some control (independent of biotype)	Warwick *et al.* (1980)
Clethodim	Some control	Finlyson *et al.* (2000)
Coldinofop	Ineffective control (in wheat)	Yu *et al.* (2014)

Cumyluron	Some control (in turf)	Askew and McNulty *et al.* (2014)
Dazomat	Reduced growth but not eradication	Branham *et al.* (2004)
Dichlobenil	Effective control (in *C. dactylon* turf)	Downing *et al.* (1970)
Dithiopyr	Reduced growth	Cross *et al.* (2012)
Diuron	Reduced growth but didn't eradicate	Lee (1981)
Endothal	Effective control	Engel *et al.* (1960)
Ethephon	Effective seed head reduction (in turfs)	Haguewood *et al.* (2013)
Ethrel	Effective control (in *A. tenuis* turf)	Jagschitz (1960)
Ethofumesate	Effective control (pre and post in *L. perenne* and other cool season turf)	Breuninger *et al.* (1993)
	Effective control (depending on regime)	Lee (1981); Coats *et al.* (1986); Baker *et al.* (2005); Cross *et al.* (2012)
	Effective control (in dormant *C. dactylon* turf)	McElroy *et al.* (2004); McCarty (2008)
Fenarimol	Ineffective	Gaul *et al.* (1987)
	Effective control (pre-emergent in turf)	Breuninger (1993)
	Some control	McCarty (2008)
Fenoxaprop-p-ethyl	Ineffective control (in wheat)	Gao *et al.* (2014)
Flazasulfuron	Effective control	Toler *et al.* (2007); Brosnan *et al.* (2010)
Flenaxaprop	Ineffective control (in wheat)	Yu *et al.* (2014)
Flucarbazone-Na	Some control (in wheat)	Gao *et al.* (2014)
Flumioxazin	Effective control (pre and post in *C. dactylon* turf)	Flessner *et al.* (2013); Reed *et al.* (2013)
	Some control	Reed *et al.* (2015)
Foramsulfuron	Effective control (but affected by cold temperatures)	Wehje *et al.* (2002); Mitra (2006); Toler *et al.* (2007)
	Effective control (in *C. dactylon* turf)	Yelverton (2003); Flessner *et al.* (2013)
	Some control	Cross *et al.* (2012)
Glufosinate	Effective control	Toler *et al.* (2007)
Glyphosate	Effective control (in dormant *C. dactylon* turf)	Yelverton (2003)
	Effective control (dependent on rate, regime and timing, in *L. perenne* turf)	Flessner *et al.* (2014)
Haloxyfop-p-methyl	Ineffective	Xiao *et al.* (2006)
Imazamox	Effective control	Gaines *et al.* (2012)
Indaziflam	Effective control	Perry *et al.* (2011); Brosnan *et al.* (2012); Henry *et al.* (2012); Brosnan *et al.* (2014)

Inorganic arsenicals	Effective control (pre and post)	Beard *et al.* (1978)
Isoproturon	Effective control (in wheat)	Gao *et al.* (2014)
Lead arsenate	Effective control (in *A. tenuis* turf)	Jagschitz (1970)
	Reduced growth (in turf)	Beard *et al.* (1978)
Linuron	Some control (dependent on biotype)	Warwick *et al.* (1980)
Magnesium-iron	Effective but shade dependent	Bell *et al.* (2004)
Maleic hydrazide	Reduced seed production (in turf but non-selective)	Engel *et al.* (1960)
Mesosuluronmethyl	Some control (in wheat)	Gao *et al.* (2014)
Mesotrione	Some control (timing dependent)	Reicher *et al.* (2011)
	Effective control (dependent on temperature and timing)	Skelton *et al.* (2012)
Methabenzthiazuron	Effective control	Pourcharesse & Lours (1970)
Metham	Effective control	Peachey *et al.* (2001)
Methiozolin	Effective control (in turf)	Flessner *et al.* (2012); Koo *et al.* (2013)
	Effective control (in *A. stolonifera* turf)	Brosnan *et al.* (2013)
	Effective control (dependent on timing, application site, also selective of various turf species)	Flessner *at al.* (2013; McCullough *et al.* (2013)
	Reduction in growth (gradual, in turf)	Askew & McNulty *et al.* (2014)
	Potential for control (in turf, also selective)	Yu *et al.* (2014)
Methyl iodine	Effective control	Ohr *et al.* (2006)
Nicrosulfuron	Shows potential for control	Sidhu *et al.* (2014)
Oxadiazon	Effective control	Bingham *et al.* (1979)
	Some control	Cross *et al.* (2012)
Paclobutrazol	Reduced growth (but non-selective)	Wu *et al.* (1992)
	Effective control (in turf)	Woosley *et al.* (2003)
	Some control	Bell *et al.* (2004)
Prodiamine	Effective control	Dernoeden (1998)
Pronamide	Effective control (pre and post in *C. dactylon* turf)	Burt *et al.* (1970)
	Reduced growth	Cross *et al.* (2012)
	Effective control	Toler *et al.* (2007); McCullough *et al.* (2012)
Prosulfalin,	Effective control	Bingham (1979)
Pyroxsulam	Effective control (in wheat)	Gao *et al.* (2014)
Quizalofop-ethyl	Ineffective	Xiao *et al.* (2006)

Rimsulfuron	Effective control (but timing and intercept dependent)	Walker *et al.* (2003); Mitra (2007); Toler *et al.* (2007); Willis *et al.* (2007)
	Some control	Cross *et al.* (2012)
Simazine	Reduced growth but didn't eradicate	Lee (1981)
	Effective control	Johnson (1982)
Sodium arsenate	Reduced growth	Engel *et al.* (1960)
Sulfosulfuron	Effective control (but non-selective in *P. pratensis* turf)	Lycan *et al.* (2005); Mitra (2007)
Tepraloxydim	Effective control	Xiao *et al.* (2006)
Tralkoxydim	Ineffective control (in wheat)	Yu *et al.* (2014)
Tri-calcium arsenate	Effective control (in *A. tenuis* turf)	Jagschitz (1970)
Trifloxysulfuron	Effective control	Mitra (2006); Toler *et al.* (2007)
Trifluralin	Effective control	Juska *et al.* (1976)
PLANT GROWTH REGULATORS		
Cultess 50W	Effective control (gradual in *A. stolonifera* turf)	Breuninger (1993)
Flurprimidol	Some control (in *A. stolonifera* turf)	Johnson *et al.* (1995)
	Effective control (gradual)	Branham & Calhoun (2005)
Mefluidide	Some control (in turf)	McMahon & Hinter (2012)
Paclobutrazol	Some control (in *Agrostis stolonifera* turf)	Johnson *et al.* (1995)
	Effective control (gradual)	Branham & Calhoun (2005)
Proturf Fertilier TGR	Effective control (gradual in *Agrostis stolonifera* turf)	Breuninger (1993)
BIOLOGICAL CONTROL		
Mycorrhizal fungi	Reduced growth	Gange (1998); Gange & Whitfield (2004)
	Some control	Hart *et al.* (2007)
	Potential for control	Whitfield *et al.* (2004)
Xanthomonas campestris pv. poannua	Effective control (in *C. dactylon* turf when applied during mowing)	Johnson (1994)
	Some control (in growth chambers but not in field)	Zhou & Neal (1995)
	Some control	Johnson *et al.* (1996); Imaizumi *et al.* (1997)
	Effective control (of biocide)	Imaizumi *et al.* (1999); Charudattan *et al.* (2000); McRae *et al.* (2000)

ECOSYSTEM MANAGEMENT		
Fertiliser manipulation	Effective control	Breuninger *et al.* (1993)
General	Ineffective when used alone	Baldwin (1993)
Mowing	Effective control	Beard *et al.* (1978); Breuninger *et al.* (1993); Beard (1996)
Nitrogen manipulation	Effective control	Beard *et al.* (1978); Beard (1996)
Soil moisture and aeration	Effective control	Beard *et al.* (1978); Beard (1996)
PHYSICAL CONTROL		
Boiling water	Some control	Beard *et al.* (1978)
Burial	Effective control	Jones *at al.* (1996)
Cutting	Effective control	Jones *at al.* (1996)
Hand-weeding	Some control (in turf) Effective on other weeds	Itoh *et al.* (1998) Pratley (2000); Monaco *et al.* (2002)
Litter application	Some control	Beard *et al.* (1978)
Mechanical control (unspecified)	Effective control	Baker *et al.* (2005)
Ploughing	Reduced growth	Swift *et al.* (1991)
Salt application	Some control	Beard *et al.* (1978)
Tillage	Some control Some control	Long (1938) (in Bond & Turner (2004)) Beard *et al.* (1978); Benvenuit and Macchia (2006)
Wormwood extract	Ineffective	Gomez de Barreda *et al.* (2011)
Flame weeding	Ineffective	Rask *et al.* (2012)
Heat treatment	Ineffective	Ascard (1995); Andreasen *et al.* (1999)
Soil solarisation	Effective control (in temperate areas at shallow depths)	Standifer *et al.* (1984); Peachey (2001)
Hot foam/steam/water	Some control	Dittrich *et al.* (2012)

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Appendix 4 Studies on the toxicity of biota relevant to Macquarie Island

Organisms	Impacts	Detail	Authors
SOIL MICROBES			
	No effect at recommended rate, effect at high rates	Glyphosate at 2 and 20 ug g^{-1} had no effect, 200 ug g^{-1} enhanced basal respiration temporarily	Wardle & Parkinson (1990)
	No effect at high rates	Glyphosate at 392 (recommended) and 3920 g a.i. ha^{-1} had no effect on microbial biomass, C mineralisation or nitrification	Olson *et al.* (1991)
	No effect	Glyphosate at 4 kg ha^{-1} did not influence the microbial variables measured	Wardle & Parkinson (1991)
	No effect	Glyphosate at 47, 94, 140 and 234 ug a.i. g^{-1} soil did not affect microbial biomass	Haney *et al.* (2000)
	No effect at recommended rate	Microbial respiration was unchanged at field rates (5-50 ug g^{-1}), but stimulated by rates 100 x higher	Busse *et al.* (2001)
	No effect	Glyphosate at the recommended rate did not affect microbial biomass or diversity	Lupwayi *et al.* (2004)
	No effect at recommended rate, minimal effect at high rates	No major changes to soil microbial structure at recommended rate (50 mg a.i. kg^{-1} soil). At 100x field rate (5000 mg a.i. kg^{-1} soil) produced an enrichment of bacteria and minimal change to fungal community	Ratcliff *et al.* (2006)
	Minor effect at high rates	Glyphosate at 10 x recommended (150 mg a.i. kg^{-1}) had minor short term effects on microbial activity, bacterial density and functional richness	Zabaloy *et al.* (2008)
	Temporary effect at high rates	Temporary effect at high rates (3.84 L a.i. ha^{-1}) but not at lower than recommended (0.48, 0.96, 1.92 L a.i. ha^{-1})	Gomez *et al.* (2009)
	No effect in short term, possible effect of long term exposure	Single exposure to glyphosate (15 or 150 mg a.i. kg^{-1}) caused only minor changes to microbial community structure/function but may have gradual effect	Zabaloy *et al.* (2012)

	No effect on most soil sat recommended rate	Glyphosate at 0.8-4 kg a.i. ha^{-1} (recommended) did not affect the microbial activity in 3 out of 4 soils	Banks *et al.* (2014)
	No effect at recommended rate	A meta-analysis revealed field application rates do not affect soil microbial biomass (< 10 mg kg) but high rates (10-100 mg kg) reduce microbial biomass	Nguyen *et al.* (2016)
AQUATIC ORGANISMS			
Microorganism s	Some effect	Mortality of algae but favoured cyanobacteria at concentration of 8 mg L^{-1}	Vera *et al.* (2010)
Phytoplankton	Effect	Glyphosate at rates of 6 and 12 mg/L affected the structure of phytoplankton and periphyton assemblages	Perez *et al.* (2007)
	Some effect, species dependent	Glyphosate was toxic to some species at rates of 6 mg L^{-1} and 60 mg L^{-1}, but not to others	Wang *et al.* (2016)
Macrophytes	Effect	Glyphosate affected a macrophyte at rates of 1 – 80 mg L^{-1} , commercial formulations were more toxic	Sobrero *et al*. (2007)
Plant	Effect at high rate	Glyphosate at 50 mg L^{-1} killed an aquatic plant but doses up to 5 mg L^{-1} had little impact	Castro *et al.* (2015)
Invertebrates General	No effect at recommended rates, effect at high rate	Roundup at recommended application rates does not affect invertebrates	Folmar *et al.* (1979)
General	Effect	Negatively affected an aquatic invertebrate at rates of 1.4 – 10.6 mg a.i. L^{-1}	Cuhra *et al* (2015)
General	No effect	Glyphosate at 2 kg 1.i. h-1 did not unduly disturb stream invertebrates	Kreutzweiser *et al.* (1989)
Amphipods	Effect	Glyphosate at rates of 0.36, 0.52, 1.08 and 2.16 mg L-1 affected biomechanical parameters and energy metabolism in amphipods	Dutra *et al.* (2011)
Crab	Effect	Glyphosate at 2.5 mg L^{-1} and 5 mg L^{-1} and Roundup formulation at 2.5 mg L^{-1} affected larvae of an estuarine crab	Avigliano *et al.* (2014)
Crustaceans	Effect	Glyphosate at rates of 1.06 mg L^{-1} and 60.97-107.53 mg L^{-1} induced 50 % mortality in a copepod and decapod shrimp, respectively	Ashoka Deepananda *et al.* (2011)

Fish (marine)	Effect	Increasing glyphosate concentrations (100, 200, 300, 400 and 500 ppm) affected survival and hatching of a tropical marine fish	Shahrizad *et al.* (2014)
Fish (marine)	No effect	Glyphosate at 0.1, 1, 10 and 100 g L^{-1} did not affect hatching and survival of a marine fish	Mer *et al.* (2013)
Macroinverteb rates	Effect	Glyphosate at a concentration of 0.09 mg dm-3 in a water body affected macroinvertebrate abundance	Ryzmski *et al.* (2013)
Mussels	Effect	Freshwater mussels were highly sensitive to glyphosate at a concentration of 0.5 mg L^{-1}	Bringolf *et al.* (2007)
Oyster	No effect at recommended rate	Glyphosate and AMPA did not affect embryo-larval development at concentrations of 0.1-1000 ug L^{-1}. LC50 was 100 000 ug L^{-1} for glyphosate and AMPA but around 6000 for commercial formulations	Mottier *et al.* (2013)
Predators	No Effect	Glyphosate at 6.5 mg a.e. L^{-1} did not affect a dragon fly or newt	Ujszegi *et al.* (2015)
TERRESTRIAL ORGANISMS Invertebrates			
Crustacean	Toxic	EC50 4.2 mg a.i. L^{-1} was toxic to Daphnia magna but around 40-50 for commercial formulations	Sihtmäe *at el.* (2013)
Beetle	Toxic	Glyphosate at a rate of 36 % p/v (360 g/L) caused 100 % toxicity	Castilla *et al.* (2010)
Earth worms	Effect	Glyphosate at the recommended field rate (6 L of formulated/ha) and double rate (12 L of formulated/ha) reduced growth of earth worms	Santadino *et al.* (2014)
Earth worms	No effect at recommended rate	Glyphosate showed no toxic effects for 2 species even at a rate of 47 mg a.i. kg^{-1}	Buch *et al.* (2013)
Earthworms, collembolans	No effect	No effect at recommended rate (30.8 % a.i.)	Santos *et al.* (2012)
Litter invertebrates	No effect	Glyphosate at 360 g a.i. L^{-1} (recommended) did not result in any direct or in-direct impacts on leaf litter invertebrates	Lindsay & French (2004)
Nematodes	No effect	Maintained or increased nematode populations at 0.75 or 1 kg a.i./ha	Patra & Ray (1987)

Predatory arthropods	Effect at recommended rate	Glyphosate at recommended field application rate (12 g/L) disrupted the behaviour of wolf spider and ground beetle	Evans *et al.* (2010)
Snails	No effect	Glyphosate at 450 g L^{-1} (4 L ha^{-1}) was not lethal to snails	Druart *et al.* (2011
Spider	No effect	Mortality was low (< 15 %) at glyphosate concentrations of 180, 360, 720, 1080, 1440, 2160 g ha^{-1}	Haughton *et al.* (2001)
Invertebrates	Effect	Glyphosate at rates of 360 g, 720 g, and 1440 g a.i. ha^{-1} reduced invertebrate abundance	Haughton *et al. (*1999)
Arthropods	No effect	Glyphosate at 7.2 g L^{-1} had no effect on soil and litter macroarthropods in rainforest	Nakamura *et al. (*2008)
Mammals			
	Affected behaviour	Glyphosate application affected small mammal behaviour due to changes in food and cover	Santilo *et al. (*1989)
	No effect	Glyphosate application induced habitat changes but did not affect the distribution and abundance of small mammals	Sullivan & Sullivan (1982)
	Some effect	Glyphosate application had some effect on moose behaviour but only due to habitat alteration	Escholz *et al. (*1996)
	Some effect	Large mammals consuming contaminated vegetation and small mammals consuming contaminated insects may be affected by high rates of glyphosate (7 lb a.i. ac^{-1}).	Bautista (2007)
Birds			
	Some effect	Birds not affected by eating contaminated vegetation. Small birds affected by consuming insects contaminated with glyphosate at a high rate	Bautista (2007)
Non-target plants			
Bryophytes and lichens	Effect	Glyphosate at recommended application rates affected bryophytes and lichens	Newmaster *et al.* (1999)
Sub-Antarctic grasses	No effect	Preliminary ex situ research suggests glyphosate at low rates does not affect native grass species	Williams *et al.* (2016)

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