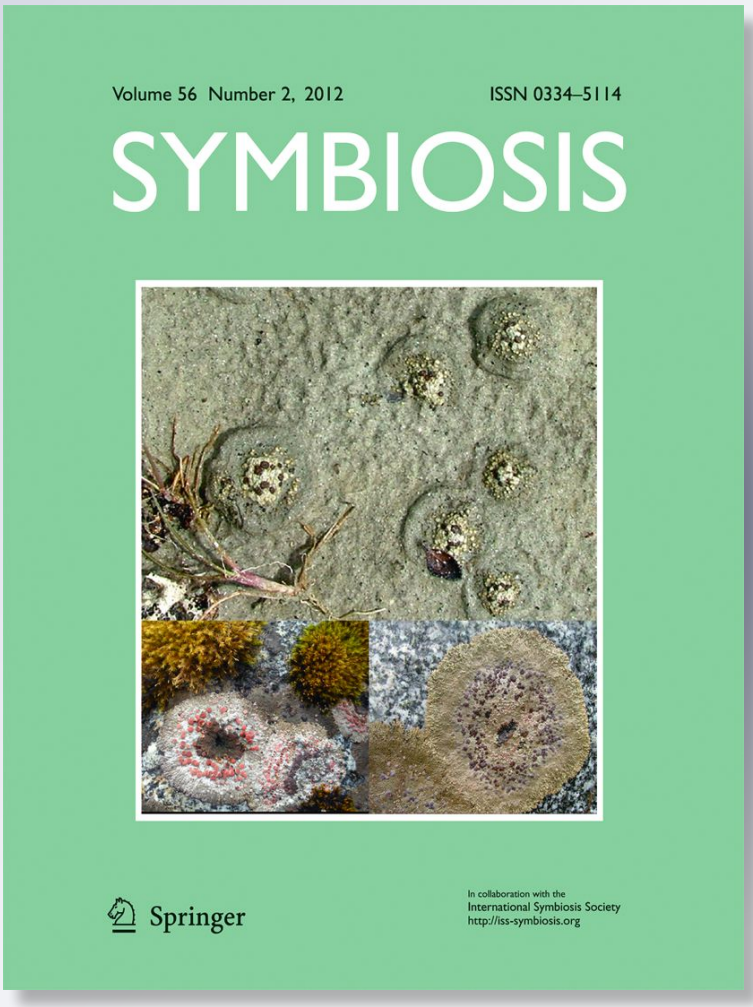
Vertebrate faeces as sources of nodulating Frankia *in Patagonia*

Eugenia E. Chaia, Maria C. Sosa & Estela Raffaele

Symbiosis

ISSN 0334-5114
Volume 56
Number 3

Symbiosis (2012) 56:139-145
DOI 10.1007/s13199-012-0169-z



Springer

Symbiosis (2012) 56:139–145
DOI 10.1007/s13199-012-0169-z

Vertebrate faeces as sources of nodulating *Frankia* in Patagonia

Eugenia E. Chaia · Maria C. Sosa · Estela Raffaele

Received: 21 November 2011 / Accepted: 18 May 2012 / Published online: 8 June 2012

Abstract *Frankia* strains nodulate the native actinorhizal plant *Ochetophila trinervis* (sin. *Discaria trinervis*), which grows in stream margins and nearby areas in northwest Patagonia (Argentina). Infective *Frankia* are found in soils with presence of host plants but also may be found in areas lacking them. This may be partly explained by water transport of *Frankia* propagules but there are other possible sources. The aim of this study was to discover whether the faeces of introduced mammalian herbivores, including cows (*Bos taurus*, adult and calf), horses (*Equus caballus*), sheep (*Ovis aries*), red and/or fallow deer (*Cervus elaphus* and *Dama dama*, respectively), wild boar (*Sus scrofa*), European hare (*Lepus capensis*), or the native upland goose (*Chloephaga picta*), could be a source of infective *Frankia*, and enhance its dispersal. Faecal material and soil samples were aseptically sampled in different plant communities, and tested *via* plant bioassays using *O. trinervis*. The faeces of all animals contained infective *Frankia* and led to an effective symbiosis with this plant. Faeces of large introduced herbivores gave rise to higher nodulation (number of nodulated plants with respect to the total number of inoculated plants) than faeces of hare and upland goose. Soils from the sites where the cow (two sites), sheep, wild boar and deer faeces were collected did not contain infective *Frankia*. This suggests that the animals may have ingested *Frankia* from plant material and that the *Frankia* propagules passed through the digestive tracts of the animals without losing its infectivity. We conclude that the faeces of large introduced herbivores contribute to the dispersal of infective *Frankia* in Northwest Patagonia.

Keywords Actinorhizal symbiosis · *Discaria* · Dispersal · Native and exotic herbivores · nitrogen fixation · *Ochetophila*

E. E. Chaia (✉)
Centro Regional Universitario Bariloche, Universidad Nacional del Comahue / INIBIOMA,
Quintral 1250,
S.C. Bariloche R8400 FRD, Argentina
e-mail: eugeniachaia@yahoo.com.ar

M. C. Sosa
Centro Regional Universitario Bariloche,
Universidad Nacional del Comahue,
Quintral 1250,
S.C. Bariloche R8400 FRD, Argentina

E. Raffaele
Laboratorio Ecotono, Universidad Nacional del Comahue-INIBIOMA/CONICET,
Quintral 1250,
S.C. de Bariloche R8400 FRD, Argentina

1 Introduction

Infective soilborne *Frankia* strains occur on all continents, except Antarctica. In some cases *Frankia* strain distribution appears to reflect the range of the plant host, like those that infect *Casuarina*. However, other strains, e.g the ones that infect *Alnus* are considered cosmopolitan and occur at sites outside the area where this host occurs (Benson et al. 2004; Benson and Dawson 2007), being found in circumpolar (Huss-Danell et al. 1999), as well as southern and central African regions (Gtari and Dawson 2011). *Frankia* strains may occur under a wide range of environmental conditions (for a review see Dawson 2008) including early successional sites prior to the establishment of any actinorhizal host. These include coastal sand-dune areas (Oremus 1980; McCray Batzli et al. 2004), a barrier island (Young et al. 1992), volcanic deposits (Burleigh and Dawson 1994a), glacial forelands (Kohls et al. 1994) and disturbed sites that have been reafforested or in degenerated forest soils after fires (Huss-Danell and Frej 1986) and mine spoils (Zimpfer et al. 1997; Densmore 2005).

The wide distribution of *Frankia* bacteria in soils might be partly explained by their mode of dispersal (Dawson 2008),

which are still not fully understood. There are uncertainties about the physiology, ecological niches and interactions of *Frankia* in soil (Chaia et al. 2010). Host plants may be the main factor in maintaining and amplifying *Frankia* populations in soil by releasing *Frankia* propagules from senescent actinorhizal nodules (Van Dijk 1984). *Frankia* strains may be able to remain viable in soils via saprophytic growth (Smolander and Sundman 1987; Hahn et al. 1999; Mirza et al. 2007, 2009) and this could contribute to short distance dispersal. The spreading of *Frankia* bacteria in soils without hosts may be via the abiotic and biotic transport of resistant propagules able to persist in the environment (Burleigh and Dawson 1994b; Burleigh and Dawson 1995; Chaia et al. 2005; Huss-Danell et al. 1997). The long distance dispersal of *Frankia* has been attributed to wind, water, animals (Bond 1976; Arveby and Huss-Danell 1988; Huss-Danell et al. 1997; Paschke 1993; Paschke and Dawson 1993) and to human activities, like the addition of soil amendments, such as leaf-mould, peat (Rodríguez-Barrueco 1968) and manure (Houwers and Akkermans 1981).

There is strong evidence to suggest that infective *Frankia* may be dispersed by some invertebrates, such as arthropods and earthworms (Paschke 1993; Redell and Spain 1991). Furthermore, Paschke and Dawson (1993) found that bird nests contained infective *Frankia*, while Burleigh and Dawson (1995) showed that *Frankia* spores were infective after passage through the digestive tract of captive parakeets (*Melopsittacus undulates)*. More recently, faecal samples directly collected from birds under natural conditions resulted in successful nodulation in *Morella cerifera* seedlings (Bissett 2008).

In northwest Patagonia, various actinorhizal plant species occur that belong to the family Rhamnaceae and are found in plant communities, including humid and xeric forests, shrublands and steppes. Infective *Frankia* bacteria have been found in many soils of the region, including those devoid of actinorhizal plants (Chaia et al. 2006). The occurrence of strains infecting *Ochetophila trinervis* (sin. *Discaria trinervis*) in soils without actinorhizal plants may due to water transport because *Frankia* has been found in sediments of glacial lakes (Chaia et al. 2005) and in wetland meadow soils in valley bottoms subjected to periodical flooding (Cardoso et al. 2010). *Ochetophila trinervis*, is a plant that has potential in the reclamation of disturbed land in this part of Patagonia (Reyes et al. 2011).

Domestic and wild mammalian herbivores were introduced in the region about a century ago (Vazquez 2002). These animals have caused a large negative impact on the structure and composition of vegetation in the native forest, shrubland and step communities (Relva and Veblen 1998; Blackhall et al. 2008; Raffaele and Veblen 2001; Raffaele et al. 2011; Nuñez et al. 2009; Barrios-Garcia et al. 2011; Vazquez 2002).

The aim of the present study was to discover if the introduced mammalian herbivores in Northwest Patagonia and the native upland goose (*Chloephaga picta*), might be sources or means of dispersal of *Frankia* propagules capable of nodulating *Ochetophila trinervis*.

2 Material and methods

The study was performed in northwestern Patagonia (41°12′ S and 71°20′ W), near to the city of San Carlos de Bariloche, Argentina. Sampling sites included forest, shrubland and steppe communities (Fig. 1, Table 1) in which any Rhamnaceae actinorhizal species (*Ochetophila trinervis*, *Discaria chacaye*, *D. articulata* and *Colletia hystrix*) occurred. The climate is temperate cool, with strong westerly winds coming from the Pacific Ocean, with maximum speeds between September and January. Mean annual temperatures are about 6–8ºC, and precipitation is mainly concentrated in winter, ranging eastward from 500 to 1000 mm yr^{-1} (Paruelo et al. 1998). Soils are Andisols, Molisols, Inceptisols and Xeric Mollisols, in forest, wetland, shrubland and steppe sites, respectively (del Valle 1998; Roig and Roig 2004; Laos et al. 2000).

Fresh faeces of sheep (*Ovis aries*), red and/or fallow deer (*Cervus elaphus* and *Dama dama*, respectively), wild boar (*Sus scrofa*), hare (*Lepus capensis*) and upland goose (*Chloephaga picta*) were aseptically collected from deposits found in the field (Table 1). Samples were placed in plastic bags. Since faeces of red and fallow deer cannot be distinguished by simple methods, results refer generically to deer (Relva and Caldiz 1998). Faecal material of cows (adults and calves) (*Bos taurus*) and horse (*Equus caballus*) were obtained by cutting each fecal pellet in two pieces with a knife, whereupon a sub-sample was collected from the inner part with a disposable spoon. At each site, three soil samples (about 500 g each) were randomly collected with a shovel at 0–15 cm depth, to use as controls to check for the occurrence of soilborne *Frankia*. Soil samples from the same site were combined. All devices used for sampling were disinfected with 1 % sodium hypochlorite and then with 70 % ethanol. Faeces and soil samples were stored at 4 °C before infectivity assays were performed.

The presence of infective *Frankia* was tested by a plant bioassay performed in test tubes filled with 30 mL of a mixture (1:1, v/v) of sterile sand and vermiculite as substrate. Seeds of *Ochetophila trinervis* (collected in Bariloche, February 2005) and dry-stored at −20ºC, were scarified and stratified and germinated on moist sterile vermiculite (Chaia et al. 2006). Two seedlings at the cotyledon stage, were transferred to each tube.

The faecal samples from each animal species were tested separately and considered as a replicate. In the laboratory, the inocula were prepared as follows: the faecal samples of sheep, deer, wild boar, hare and upland goose were

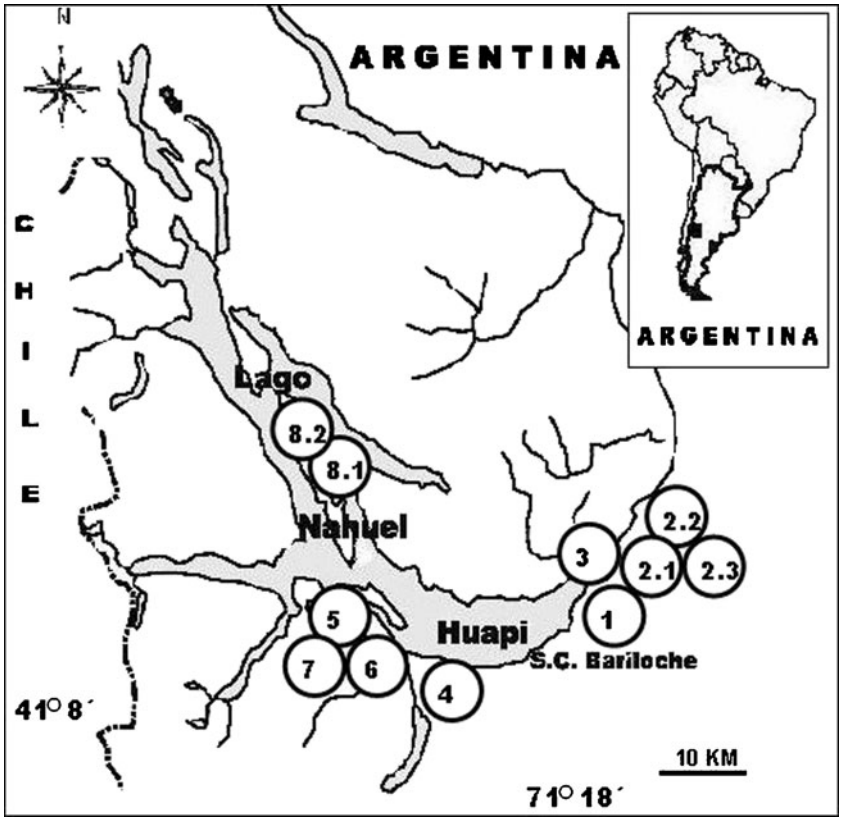


Fig. 1 Schematic map of the sampling sites of Northwest Patagonia where faeces of different vertebrates and soils were collected (see site codes and faeces origin in Table 1)

aseptically cut with a scalpel, and a sub-sample was collected with another sterile scalpel from the inner part, taking care to avoid collecting superficial parts which could have been in contact with the soil. 1 to 3 g (fresh weight) of each fecal sub-sample was added to each sterile test tube containing 10 mL of Evans nutrient solution without N diluted to 1 / 10 of full strength (Huss-Danell 1978) and mixed in a vortex for 1 min. Due to the exploratory character of this study, different inoculum doses and growths conditions were used in assays (see Table 2). For each replicate, one to three tubes with seedlings were used and inoculated with a faeces suspencion dose of 1 mL. Additionally, the faeces of horse, wild boar, deer, upland goose and hare were inoculated to other tubes with plants at a dose of 2 mL (Table 2). To

Table 1 Faeces of different vertebrates collected at sites of Northwest Patagonia (see site location in Fig. 1) used to test the presence of *Ochetophila trinervis* infective *Frankia*

Faeces source	Site	Site code	Plant community
Cow	Ñirihuau	1	Shrubland
Cow	San Ramón	2.1	Steppe
Cow	Paso Flores	3	Shrubland
Calf	San Ramón	2.3	Wetland meadow
Sheep	Paso Flores	3	Shrubland
Sheep	San Ramón	2.2	Steppe
Horse	Laguna Fantasma	5	Wetland meadow
Horse	Bahía Serena	6	Wetland meadow
Horse	Hipódromo	7	Shrubland
Wild Boar	Isla Victoria, Pen. Manzanito	8.1	Forest
Wild Boar	Isla Victoria, Centro	8.2	Forest
Deer	Isla Victoria, Pen. Manzanito	8.1	Forest
Deer	Isla Victoria, Centro	8.2	Forest
Hare	Virgen de las Nieves	4	Shrubland
Upland Goose	Bahía Serena	6	Wetland meadow

Table 2 Vertebrate faeces inoculated to *Ochetophila trinervis* seedlings grown in test tubes. Mean (± standard deviation) inoculum dose (on a dry weight basis) diluted in Evans solution N-free 1 / 10 of full strength. Site codes are described in Table 1

Faeces source (site code)	Number of faeces tested (replicates)	Faeces inoculum dose (mg/mL)	Inoculum dose/s applied to each tube (mL)	Test tubes inoculated with each dose
Cow (1)	3	276	1	3
Cow (2.1)	10	150±51	1	1
Cow (3)	5	278	1	3
Calf (2.3)	5	151±25	1	1
Sheep (3)	5	42±1	1	3
Sheep (2.2)	15	212±59	1	1
Horse (5)	2	271±9	1 / 2	2
Horse (6)	5	263±11	1 / 2	2
Horse (7)	3	259±5	1 / 2	2
Wild Boar (8.1)	8	264±14	1 / 2	2
Wild Boar (8.2)	2	200±84	1 / 2	2
Deer (8.1)	4	204±36	1 / 2	2
Deer (8.2)	10	256±26	1 / 2	2
Hare (4)	10	98±2	1 / 2	2
Upland Goose (6)	5	96±3	1 / 2	2

determine the occurrence of infective *Frankia* in soils of the collection site, seedlings were inoculated with 2 g of each soil. One to three additional tubes per treatment were also inoculated with *Frankia* strain BCU110501 (Chaia 1998) to be used as positive controls and an additional 10 to 20 pots or tubes with only substrate and seedlings served as negative control, for each assay. All tubes were capped with sterile cotton plugs and their lower parts were protected from light.

At the beginning of the assays seedlings were watered with Evans solution diluted to one tenth of full strength and with N 0.71 mM as NH_4NO_3 (Huss-Danell 1978). Then, seedlings were watered when necessary and cultivated for 10 to 12 weeks. Plants inoculated with samples from Ñirihuau and Paso Flores sites (Fig. 1) were grown in a greenhouse, with 16 h daylight and without temperature and humidity control. Plants inoculated with all other inocula were grown in a growth chamber with 16 h photoperiod provided by metal halogen lamps (Philips HPI-T 400 W and Philips SON-T Plus 400) (photosynthetically active radiation was ca. 318 μM m^{-2} s^{-1}). Average minimum and maximum temperatures were 22 and 27 °C, respectively, while average relative humidity was 44 %. Subsamples of faeces and soils were placed in the drying oven at 70ºC for 24 h to further determine their water content. Wet and dry weights were measured before and after drying, respectively. The water content was calculated from the wet and dry weights.

The plant growth and the presence of nodulation were recorded in every plant. Actinorhizal nodules were fixed in 4 % glutaraldheyde. Lobes of 3 nodules per plant were excised in slices by hand, stained with cotton blue and were examined under an Olympus light microscope to determine the presence of *Frankia* vesicles.

The nodulation was recorded as the number of nodulated plants with respect to the total number of inoculated plants. Data were calculated as average number nodules per plant within each tube. The mean growth of nodulated *O. trinervis* seedlings inoculated with faeces was compared with the non-inoculated control corresponding to the same assay by Mann-Whitney *U*-Test .

3 Results and discussion

In nature, interactions occur among actinorhizal organisms and a dynamic range of biotic, physical and chemical agents. Such complexity is the norm and not the exception. A complex of environmental factors determines the actual survival, growth nodulation and nitrogen fixation of the symbiotic partners at any given site on the Earth's surface (Dawson 2008). This study provides one example of these interactions between introduced mammalian herbivores (and a native bird) and the nitrogen fixing actinomycete *Frankia* in various vegetation communities of Northwest Patagonia.

Our results showed that the faeces of the different vertebrates collected in the field (Tables 1 and 3) contained *Frankia* that was able to infect *Ochetophila trinervis*, a plant in the Rhamnaceae. However, this ability varied with the different animals; faeces of the large herbivores (cow and calf, sheep, horse, deer and wild boar), produced more nodulated plants than the faeces of hare and the native upland goose (Table 3). The nodulating strains were able support an effective symbiosis as indicated by the green shoots, the presence of vesicles inside the nodules and the

Table 3 Nodulation of *Ochetophila trinervis* plants (number of nodulated plants / total number of inoculated plants) inoculated with vertebrate faeces or soils. Positive controls were additionally inoculated with *Frankia* BCU110501. Site codes are described in Table 1

Faeces source (site code)	Inoculum			
	Faeces		Soil	
	Treatment	Positive control	Treatment	Positive control
Cow (1)	1 / 9	0 / 9	0 / 3	2 / 3
Cow (2.1)	8 / 10	8 / 10	3 / 7	5 / 7
Cow (3)	2 / 15	5 / 15	0 / 3	2 / 3
Calf (2.3)	5 / 5	4 / 5	0 / 7	4 / 7
Sheep (3)	2 / 15	4 / 15	0 / 3	2 / 3
Sheep (2.2)	14 / 15	13 / 15	0 / 7	4 / 7
Horse (5)	3 / 4	2 / 4	nd[a]	nd
Horse (6)	5 / 10	8 / 10	4 / 7	7 / 7
Horse (7)	5 / 6	4 / 6	1 / 7	0 / 7
Wild Boar (8.1)	13 / 16	15 / 16	0 / 7	7 / 7
Wild Boar (8.2)	4 / 4	4 / 4	2 / 7	5 / 7
Deer (8.1)	1 / 8	5 / 8	2 / 7	5 / 7
Deer (8.2)	10 / 20	13 / 20	0 / 7	7 / 7
Hare (4)	1 / 20	7 / 20	4 / 7	4 / 7
Upland Goose (6)	1 / 10	6 / 10	4 / 7	7 / 7

[a] na: not determined

high dry weight of the nodulated plants (Table 4). The negative control plants did not develop any nodules.

Although we included controls to try to detect any *Frankia* contamination of the faeces by the surrounding soil (soil controls), it is not possible to rule out entirely contamination from the environment. Inoculation with hare and upland goose faeces resulted in extremely low nodulation, and the soils from the same sites were infective. Thus, it is possible that those faeces were contaminated with soils. However, in the case of faeces of cow, sheep, calf, deer and wild boar, we can disregard the notion of *Frankia* propagules originating from soil contamination. This is because none of the soil samples collected from the same sites as the faeces were infective (Table 3). Therefore it is likely that these animals are transporting agents of *Frankia* as a result of ingestion of propagules. Horse faeces may also be transporting agents, but more studies are necessary to confirm this because the associated soils also contained *Frankia* and were infective in the bioassay. Indirect evidence that cattle faeces are a significanat sources of *Frankia* propagules is also provided by the finding that non-inoculated *Alnus glutinosa* seedlings grown for 2 years in plots treated with farmyard manure were highly nodulated as compared with seedlings treated with P, K and N amendments (Houwers and Akkermans 1981).

Some of the herbivores in Patagonia consume actinorhizal shrubs. For example, red and fallow deer browse *Colletia hystrix*, *Discaria chacaye* (Relva and Caldiz 1998; Relva and Veblen 1998, Barrios-Garcia et al. 2011) and *D. articulata* (Pelliza et al. 1997), while cows and sheep have been observed to feed on *D. chacaye* and *D. articulata* (Manacorda et al. 1996). We suggest that *Frankia* propagules, in the form of spores or hyphae, may be present on the surfaces of these plants that are close to the soil surface. It is known that infective *Frankia* occurs in the superficial soil layers (at 0–3 cm soil depth) in the region (Chaia and Raffaele 2000). Therefore it is be possible that propagules are ingested during

Table 4 Mean growth and nodulation (and standard deviation) of *O. trinervis* seedlings inoculated and nodulated with vertebrate faeces collected in field in Northwest Patagonia. Site codes are described in Table 1. Significant differences with the non-inoculated control corresponding to the same assay are indicated by an asterisk ($P<0.05$)

Inoculum (Site code)	N	Nodules per plant (number)	Shoot Height (cm)	Root Length (cm)	Shoot DW (mg)	Shoot/ Root (DW)
Cow (1)	1	1	13.0	11.5	9.4	1.4
Cow (2.1)	8	11±14	3.8±1.0	13.0±1.6	9.6±2.3 *	0.5±0.1
Cow (3)	2	2	14.8±1.1	13.2±2.5	9.1±2.8	0.7±0.4
Calf (2.3)	5	7±2	3.9±1.0	12.6±2.2	9.9±3.2 *	0.6±0.3
Sheep (3)	2	2	10.5±3.5 *	12.0±2.8	13.0±9.2 *	0.9±0.1 *
Sheep (2.2)	14	12±10	9.5±3.2 *	13.4±1.6	16.7±4.3 *	0.7±0.2 *
Horse (5)	3	3±3	4.3±1.8	13.3±5.6	8.1±5.5	0.6±0.2
Horse (6)	5	5±8	4.5±1.0	13.7±1.5	10.5±3.1 *	0.4±0.1
Horse (7)	5	10±8	6.1±1.2	13.5±0.9	17.9±3.6 *	0.6±0.1
Wild Boar (8.1)	13	10±8	9.3±5.4 *	14.5±2.0 *	19.1±5.9 *	0.9±0.4 *
Wild Boar (8.2)	4	38±24	13.3±3.2 *	11.4±2.1	27.5±8.4 *	1.0±0.4 *
Deer (8.1)	1	7	2.6	17.2	6.7	0.6
Deer (8.2)	10	5±3	3.6±1.3	14.8±2.2	6.2±3.0 *	0.8±0.4
Hare (4)	1	5	4.7	15.0	9.2	0.5
Upland Goose (6)	1	1	3.8	10.5	25.0	0.7

grazing (cow, sheep and calf) or browsing (sprouts, saplings, leaves, or bark) at or near ground level (deer, horse and cow). Digging and rooting activities by wild boar could be the source of their *Frankia* propagules. It appears that all or a part of consumed *Frankia* propagules remain infective after passage through the digestive tract of mammals. Thus, Burleigh and Dawson (1995) demonstrated that the spores of a *Frankia* strain survived passage through the digestive tracts of captive parakeets but hyphal filaments lost most of their infective capacity. It would be interesting to study if scarification of *Frankia* propagules occurs during passage through the digestive tract of mammals and whether this might increase the nodulation capacity to a different extent depending on the animal species.

Spores of other symbiotic plant microbes like the vesicular-arbuscular mycorrhizal fungi *Glomus* have been already identified in the faeces of wild mammals such as elk (*Cervus elaphus nelsoni*), mule deer (*Dama hemionus hemionus*), and pronghorn antelope (*Antilocapra americana*) (Warner et al. 1987). However this paper appears to be the first to report herbivore mammal species as a significant source of infective *Frankia*. The finding that the faeces of some large introduced mammals in Patagonia contain and may disperse infective *Frankia*, indicates an impact on local ecosystems. The role of these exotic vertebrates should be considered in studies on the spread of soil microorganisms such as the symbiotic nitrogen fixing bacterium *Frankia*.

Acknowledgments We thank Mark Paschke for early comments and suggestions for performing this study, to Andrea Relva and Gladys Galende for help in sampling and faeces identification and to David Richardson and three anonymous referees that contributed to improve the quality of the paper. This work was funded by Universidad Nacional del Comahue and Agencia Nacional de Promoción Científica y Tecnológica.

References

Arveby AS, Huss-Danell K (1988) Presence and dispersal of infective *Frankia* in peat and meadow soils in Sweeden. Biol Fertil Soils 6:39–44

Barrios-Garcia MN, Relva MA, Kitzberger T (2011) Patterns of use and damage by exotic deer on native plant communities in Northwestern Patagonia. Eur J Wildl Res. doi:10.1007/s10344-011-0554-6

Benson DR, Dawson JO (2007) Recent advances in the biogeography and genecology of symbiotic *Frankia* and its host plants. Physiol Plant 130:318–330

Benson DRB, Vanden Heuvel B, Potter D (2004) Actinorhizal symbioses: diversity and biogeography. In: Gillings M, Holmes A (eds) Plant microbiology. BIOS Scientific Publishers, Oxford, pp 97–127

Bissett NS (2008) Avian dispersal of the Actinomycete *Frankia* across a barrier island landscape. Master of Science Thesis. Virginia Commonwealth University. Richmond, Virigina. http://amazon.evsc.virginia.edu/thesis/SPENCER_NATHANIEL_BISSETT2008.pdf Accessed 29/09/11

Blackhall M, Raffaele E, Veblen TT (2008) Cattle affect early post-fire regeneration in a *Nothofagus dombeyi—Austrocedrus chilensis* mixed forest in northern Patagonia, Argentina. Biol Conserv 141:2251–2261

Bond G (1976) The results of the IBP survey of root-nodule formation in non-leguminous angiosperms. In: Nutman PS (ed) Symbiotic nitrogen fixation in plants. Cambridge University Press, Cambridge, pp 443–474

Burleigh SH, Dawson JO (1994a) Occurrence of *Myrica*-nodulating *Frankia* in Hawaiian volcanic soils. Plant Soil 164:283–289

Burleigh SH, Dawson JO (1994b) Desiccation tolerance and trehalose production in *Frankia* hyphae. Soil Biol Biochem 25:593–598

Burleigh HB, Dawson JO (1995) Spores of *Frankia* strain HFPCc13 nodulate *Casuarina equisetifolia* after passage through the digestive tracts of captive parakeets (*Melopsittacus undulatus*). Can J Bot 73:1527–1530

Cardoso BM, Chaia EE, Raffaele E (2010) Are Northwestern Patagonian "mallín" wetland meadows reservoirs of *Ochetophila trinervis* infective *Frankia*? Symbiosis 52:11–19

Chaia EE (1998) Isolation of an effective strain of *Frankia* from nodules of *Discaria trinervis* (Rhamnaceae). Plant Soil 205:99–102

Chaia E, Raffaele E (2000) Spatial patterns of root branching and actinorhizal nodulation in *Discaria trinervis* seedlings. Symbiosis 29:329–341

Chaia EE, Ribeiro Guevara S, Rizzo A, Arribére M (2005) Occurrence of *Discaria trinervis* nodulating *Frankia* in dated sediments of glacial Andean lakes. Symbiosis 39:67–75

Chaia EE, Fontenla S, Vobis G, Wall LG (2006) Infectivity of soilborne *Frankia* and micorrhizae in *Discaria trinervis* along a vegetation gradient in Patagonian soil. J Basic Microbiol 46:263–274

Chaia EE, Wall LG, Huss-Danell K (2010) Life in soil by the actinorhizal root nodule endophyte *Frankia*. A review. Symbiosis 51:201–226

Dawson JO (2008) Ecology of actinorhizal plants. In: Pawloski K, Newton WE (eds) Nitrogen fixation: origins, applications, and researh progress, Vol 6, nitrogen-fixing actinorhizal symbioses. Springer, Dordrecht, pp 199–234

del Valle H (1998) Patagonian soils: a regional synthesis. Ecol Austral 8:103–123

Densmore RV (2005) Succession on subalpine glacier mine spoil: effects of revegetation with *Alnus viridis*, Alaska, U.S.A. Arctic Antarct Alpine Res 37:297–303

Gtari M, Dawson JO (2011) An overview of actinorhizal plants in Africa. Funct Plant Biol 38:653–661

Hahn D, Nickel A, Dawson J (1999) Assessing *Frankia* populations in plants and soil using molecular methods. FEMS Microbiol Ecol 29:215–227

Houwers A, Akkermans ADL (1981) Influence of inoculation on yield of *Alnus glutinosa* in the Netherlands. Plant Soil 61:189–202

Huss-Danell K (1978) Nitrogenase activity measurements in intact plants of *Alnus incana*. Physiol Plant 43:372–376

Huss-Danell K, Frej A (1986) Distribution of *Frankia* in soils from forests and afforestation sites in Northern Sweden. Plant Soil 90:407–418

Huss-Danell K, Uliassi D, Renberg I (1997) River and lake sediments as sources of infective *Frankia* (*Alnus*). Plant Soil 197:35–39

Huss-Danell K, Sverrisson H, Hahlin AS, Danell K (1999) Occurrence of *Alnus*-infective *Frankia* and *Trifolium* infective *Rhizobium* in circumpolar soils. Arctic Antarct Alpine Res 31:400–406

Kohls SJ, Van Kessel C, Baker DD, Grigal D, Lawrence DB (1994) Assessment of N_2 fixation and N cycling by *Dryas* along a chronosequence within the forelands of the Athabasca glacier, Canada. Soil Biol Biochem 26:623–632

Laos F, Satti P, Walter I, Mazzarino MJ, Moyano S (2000) Nutrient availability of composted and noncomposted residues in a Patagonian Xeric Mollisol. Biol Fertil Soils 31:462–469

Manacorda M, Somlo M, Sbriller AP, Willems P (1996) Dieta de ovinos y bovinos en la región de los bosques de ñire (*Nothofagus antartica*) de Río Negro y Neuquén. RIA 26:137–146

McCray Batzli J, Zimpfer JF, Huguet V, Smyth CA, Fernandez M, Dawson JO (2004) Distribution and abundance of infective, soilborne *Frankia* and host symbionts *Shepherdia, Alnus* and *Myrica* in a sand dune ecosystem. Can J Bot 82:700–709

Mirza BS, Welsh A, Hahn D (2007) Saprophytic growth of inoculated *Frankia* sp. in soil microcosms. FEMS Microbiol Ecol 62:280–289

Mirza BS, Welsh A, Rieder JP, Paschke MW, Hahn D (2009) Diversity of frankiae in soils from five continents. Syst Appl Microbiol 32:558–570

Nuñez MA, Bailey JK, Schweitzer JA (2009) Population, community and ecosystem effects of exotic herbivores: a growing global concern. Biol Invasions 12:297–301

Oremus PAI (1980) Occurrence and infective potential of the endophyte of *Hippophae rhamnoides* L. ssp. *rhamnoides* in coastal sand-dune areas. Plant Soil 56:123–139

Paruelo JM, Beltrán A, Jobbágy E, Sala OE, Golluscio RA (1998) The climate of Patagonia: general patterns and controls on biotic processes. Ecol Austral 8:85–101

Paschke MW (1993) Distribution and dispersal of *Frankia*. Ph.D. Thesis. University of Illinois at Urbana-Champaign. Illinois, USA, pp 83

Paschke MW, Dawson JO (1993) Avian dispersal of *Frankia*. Can J Bot 71:1128–1131

Pelliza A, Willems P, Nakamatsu V, Manero A (coordinadores) (1997) Atlas dietario de herbívoros patagónicos. In: Somlo R (ed) INTA Estación Experimental Agropecuaria Bariloche, pp. 109

Raffaele E, Veblen TT (2001) Effects of cattle grazing on early postfire regeneration of matorral in northwest Patagonia, Argentina. Nat Areas J 21:243–249

Raffaele E, Veblen TT, Blackhall M, Tercero-Bucardo N (2011) Synergistic influences of introduced herbivores and fire on vegetation change in northern Patagonia, Argentina. J Veg Sci 22:59–71

Redell P, Spain AV (1991) Transmission of infective *Frankia* (actinomycetales) propagules in casts of the endogeic earth-worm *Pontoscolex corethurus* (Oligochaeta, Glossoscolecidae). Soil Biol Biochem 23:775–778

Relva MA, Caldiz MS (1998) Composición estacional de la dieta de ciervos exóticos en Isla Victoria, Parque Nacional Nahuel Huapi, Argentina. Gayana Zool 62:101–108

Relva MA, Veblen TT (1998) Impact of introduced large herbivores on *Austrocedrus chilensis* forest in northern Patagonia, Argentina. For Ecol Manage 108:27–40

Reyes MF, Gobbi ME, Chaia EE (2011) Reproductive ecology of *Ochetophila trinervis* in northwest Patagonia. Funct Plant Biol 38:720–727

Roig C, Roig FA (2004) Consideraciones generales. In: Blanco DE, de la Balze VM (eds) Los Turbales de la Patagonia: Bases para su inventario y la conservación de su biodiversidad. Publicación No. 19. Wetlands International. Buenos Aires, Argentina, pp. 29

Rodriguez-Barrueco C (1968) The occurrence of the root-nodule endophyte of *Alnus glutinosa* and *Myrica gale* in soils. J Gen Microbiol 52:189–194

Smolander A, Sundman V (1987) *Frankia* in acid soils devoid of actinorhizal plants. Physiol Plant 70:297–303

Van Dijk C (1984) Ecological aspects of spore formation in the *Frankia-Alnus* symbiosis. PhD Thesis, Leiden State University, The Netherlands, pp. 154

Vázquez DP (2002) Multiple effects of introduced mammalian herbivores in a temperate forest. Biol Invas 4:175–191

Warner NJ, Allen MF, Macmahon JA (1987) Dispersal agents of vesicular-arbuscular mycorrhizal fungi in a disturbed arid ecosystem. Mycologia 79:721–730

Young DR, Sande E, Peters G (1992) Spatial relationships of *Frankia* and *Myrica cerifera* on a Virginia, USA Barrier Island. Symbiosis 12:209–220

Zimpfer JC, Smyth CA, Dawson JO (1997) The capacity of Jamaican mine spoils, agricultural and forest soils to nodulate *Myrica cerifera, Leucaena leucocephala* and *Casuarina cunninghamiana*. Physiol Plant 99:664–672