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# Species-specific effects of polyploidisation and plant traits of *Centaurea maculosa* and *Senecio inaequidens* on rhizosphere microorganisms

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**Abstract** Invasive plant species represent a threat to terrestrial ecosystems, but their effects on the soil biota and the mechanisms involved are not yet well understood. Many invasive species have undergone polyploidisation, leading to the coexistence of various cytotypes in the native range, whereas, in most cases, only one cytotype is present in the introduced range. Since genetic variation within a species can modify soil rhizosphere communities, we studied the effects of different cytotypes and ranges (native diploid, native tetraploid and introduced tetraploid) of *Centaurea maculosa* and *Senecio inaequidens* on microbial biomass carbon, rhizosphere total DNA content and bacterial communities of a standard soil in relation to plant functional traits. There was no overall significant difference in microbial biomass between cytotypes. The variation of rhizosphere total DNA content and bacterial community structure according to cytotype was species specific. The rhizosphere DNA content of *S. inaequidens* decreased with polyploidisation in the native range but did not vary for *C. maculosa*. In contrast, the bacterial community structure of *C. maculosa* was affected by polyploidisation and its diversity increased, whereas there was no significant change for *S. inaequidens*. Traits of *S. inaequidens* were correlated to the rhizosphere biota. Bacterial diversity and total DNA content were positively correlated with resource allocation to belowground growth and late flowering, whereas microbial biomass carbon was negatively correlated to investment in reproduction. There were no correlations between traits of the cytotypes of *C. maculosa* and corresponding rhizosphere soil biota. This study shows that polyploidisation may affect rhizosphere bacterial community composition, but that effects vary among plant species. Such changes may contribute to the success of invasive polyploid genotypes in the introduced range.

**Keywords** Bacterial community structure · Invasive species · Microbial biomass carbon · Plant–soil interactions · T-RFLP

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## Introduction

Invasive species represent a major threat to natural and managed ecosystems (Pimentel et al. 2000; Prieur-Richard

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and Lavorel 2000). Therefore, understanding how they can invade ecosystems is a major challenge to ecologists (Richardson and Pysek 2006). Most work on invasion ecology has focused on the aboveground compartment (Levine et al. 2003; Batten et al. 2006). However, soil organisms play an important role in regulating ecosystem-level processes, and the aboveground and belowground compartments are tightly linked (Wardle et al. 2004). Indeed, soil microorganisms can strongly influence plant community dynamics and may contribute to the coexistence of competitive plant species or to the competitive dominance of one plant species over another (van der Heijden et al. 1998; Bever 2003; Wardle et al. 2004). Thus, changes in the soil biota could affect the resistance of plant communities to invasive species through, for example, changes in productivity, plant community composition or ecosystem functions (Klironomos 2002).

Plant–soil feedback can be highly species specific (Van Der Putten 2003). Within species, rhizosphere communities in terms of soil microbial biomass and microbial community composition can be modified by genetic variation (Schweitzer et al. 2008). Different genotypes of some species are able to create different environments to which soil microorganisms respond, leading to the selection of particular soil microbial characteristics (Schweitzer et al. 2008). Recently, there has been considerable interest in the role of evolutionary processes, such as polyploidisation or hybridisation, in promoting invasion (Lee 2002; Müller-Schärer et al. 2004; Suarez and Tsutsui 2008). Many invasive species have undergone polyploidisation in the native range (Verlaque et al. 2002; Pandit et al. 2006), leading to the coexistence of multiple cytotypes in this range, whereas in most cases only one ploidy level is present in the introduced range (Lafuma et al. 2003; Kubatova et al. 2008; Schlaepfer et al. 2008; Treier et al. 2009). While the effect of polyploidisation on aboveground traits has been investigated in recent years, nothing is yet known about the influence of polyploidisation on rhizosphere communities. Polyploidisation may have effects on rhizosphere communities (bacteria, fungi, protists, and/or invertebrates) and soil functioning (e.g. nutrient cycling), and these might be a key factor contributing to the success of an invasive species in the introduced range.

As an evolutionary process, polyploidisation can lead to changes in trait trade-offs (Blossey and Notzold 1995; Bossdorf et al. 2004). Plant functional traits are known to influence ecosystem processes (Chapin et al. 2000; Loreau et al. 2001). Plants can strongly influence the activity of soil organisms (Hobbie 1992) via their ecophysiological traits, such as nutrient use, leaf properties, and carbon allocation strategy (Cornelissen 1996; Cornelissen et al. 2003; Scherer-Lorenzen 2008). Leaf properties and nutrient use strategy can be assessed by the specific leaf area of the individual (Cornelissen et al. 2003). Since a plant allocates resources either to vegetative growth (belowground or aboveground) or reproduction, measuring root biomass, root–shoot ratio, and flowering output (time of initial flowering and flowering potential) allows the carbon investment strategy to be assessed. However, an understanding of the effects of changes in plant traits on rhizosphere community composition is still lacking (De Deyn et al. 2008).

In this study, we used two invasive species, *Centaurea maculosa* Lam. and *Senecio inaequidens* D.C., which we defined as being taxonomically related since (1) they belong to the same family (Asteraceae), (2) they occupy similar ecological niches in their native range, and (3) they tend to invade similar habitats in their introduced range. Furthermore, these two species have undergone polyploidisation in their native range, leading to the presence of diploid and tetraploid genotypes, while the introduced range is strongly if not exclusively dominated by tetraploid genotypes (Lafuma et al. 2003; Treier et al. 2009). If we define for convenience a geocytotype as a ploidy level in a given area (native or introduced range), both model species have three geocytotypes: (1) native diploid, (2) native tetraploid, and (3) introduced tetraploid.

We performed a nine-month greenhouse experiment in standard "naive" soil to test for effects of geocytotypes on rhizosphere communities. Given the fact that rhizosphere microorganisms can be influenced by the identity of the plant species as well as genetic variation within species, we hypothesised that differences in rhizosphere biota characteristics (microbial biomass carbon, total DNA content, bacterial diversity as expressed as the Shannon–Weaver diversity index) and bacterial communities (characterised by bacterial T-RFLP profiles) are due to geocytotypes, in addition to being species specific. Since plant traits are known to vary according to genetic variation and to influence ecosystem functioning via changes in soil microbial composition, we further hypothesised that differences in rhizosphere characteristics and bacterial community profiles among geocytotypes are linked to trade-offs in plant functional traits.

## Materials and methods

### Model species

*Centaurea maculosa* Lam. is a herbaceous biennial or short-lived perennial tap-rooted forb with a rosette of basal leaves and relatively thick flowering stems (Hook et al. 2004). Its native range spans from Western Asia to Western Europe. The species was introduced in the Pacific northwest of the United States in the late 1800s (Watson and

Renney 1974) and expanded rapidly throughout northwestern United States and western Canada, where it is now widespread in rangelands, pastures, and on roadsides (Skinner et al. 2000; Duncan 2001). *C. maculosa* has been demonstrated to be allelopathic (Ridenour and Callaway 2001). This species is known for its release of (±) catechin into the soil (Bais et al. 2002), and catechin has been thought to be responsible for allelopathic effects, although recent arguments claim that the amount released into the soil is too small to be able to induce allelopathic effects (Blair et al. 2006; Perry et al. 2007).

*Senecio inaequidens* D.C. is an erect perennial dwarf shrub native to South Africa and Lesotho. Originally found in grasslands from 1400 to 2800 m a.s.l., it is now common in South Africa. It was accidentally introduced into Europe at the end of the 1880s (Bornkamm 2002), where it is now widespread, even at lower altitudes. *Senecio inaequidens* contains pyrrolizidine alkaloids (Macel and Vrieling 2003), which are toxic to livestock (Noble et al. 1994). Invasive genotypes have higher pyrrolizidine alkaloid concentrations than native ones (Cano et al. 2009). The only existing study on the impact of *S. inaequidens* on the soil did not reveal any negative soil feedback (Engelkes et al. 2008). However, no study has been done as yet to test for its impact on soil communities.

### Seed collection

Seeds of *C. maculosa* were collected during summer 2005 throughout its native range in Europe as well as in Montana and Oregon, United States (Broennimann et al. 2007; Treier et al. 2009). Seeds of *S. inaequidens* (mostly provided by Dr. Daniel Prati, University of Leipzig-Halle and Sandrine Maurice, Institut des Sciences de l'Evolution Montpellier) were collected in Western Europe, South Africa and Lesotho (Lafuma et al. 2003).

### Pot experiment

A nine-month pot experiment was set up in the greenhouse. During February 2006, 180 seeds of each model species were sown in germination trays filled with sieved garden soil from the University of Lausanne which had experienced neither *C. maculosa* nor *S. inaequidens*. This choice allowed us to get a sample of the natural naive soil community that would avoid possible effects of coevolution between the soil biota and a particular geocytotype of the two studied species. Seeds were not sterilised before the experiment to allow possible colonisation of the soil by seed-borne organisms. For each species, half of the seeds were from invasive tetraploid populations and half were from native populations, either diploid or tetraploid. After one month, surviving seedlings (330 out of 360) were transplanted into 1-L pots. Each pot was filled with standard potting soil (mix of sand, peat, and Swiss garden compost made of branches and leaves without any additional microorganisms, provided by Ricoter S.A., Aarberg, Switzerland) and contained one individual. The composition of this standard potting soil prevented any possible coevolution between geocytotypes and soil microorganisms. Pots were randomly arranged on tables and watered every 2–4 days. For biosecurity reasons, and in order to prevent seed set, capitula of all plants were cut weekly during the experiment. Nine months after sowing, seven replicates of each geocytotype of the two model species were selected randomly among the pool of living individuals to measure plant traits, rhizosphere characteristics and bacterial community composition. Details on the origins of the seeds used for this study are given in Table S1 in the "Electronic Supplementary Material".

### Plant trait measurements

Two reproductive traits were monitored during the experiment to measure the *onset of flowering* and the *capitulum production*, respectively: (1) the number of days between sowing and the appearance of the first capitulum, and (2) the total number of capitula produced by flowering plants during the experiment. Five leaves were taken randomly from each individual among the healthy, fully developed leaves for measurements of total leaf area (Cornelissen et al. 2003). Total leaf area was measured using a LI-3100C Leaf Area Meter (Li-COR, Lincoln, NE, USA). Leaves were dried at 60°C for 72 h and weighed for dry mass. *Specific leaf area* (*SLA*, leaf area per unit of leaf dry mass in $m^2\ kg^{-1}$) was calculated for each plant as the mean of measurements from the five harvested leaves, and was used as a proxy for nutrient use strategy (Cornelissen et al. 2003). Plants were then harvested entirely and separated into *shoots* and *roots*, dried at 35°C for 7 days (to allow potential chemical analyses), and weighed together with the leaves collected for leaf trait measurements. *Root–shoot ratio* (*RSR*) was calculated as the ratio between root and shoot dry mass.

### Microbial measurements

Microbial carbon biomass was assessed using chloroform fumigation–$K_2SO_4$ extraction (Brookes 1985; Vance et al. 1987). Two fresh soil samples of 25 mg were prepared. Briefly, one of each pair of soil samples was fumigated with $CHCl_3$ for 24 h at 25°C. After removing the $CHCl_3$, the soluble C was extracted from fumigated and unfumigated samples with 25 ml of a 0.5 M solution of $K_2SO_4$ for an hour on an orbital shaker. Total organic C in filtered extracts (Whatman No. 1, Whatman, Clifton, NJ, USA)

was determined using a total organic carbon analyser (Shimadzu TOC 5000, Shimadzu, Kyoto, Japan). Microbial C flush (difference between extractable C from fumigated and unfumigated samples) was converted to microbial biomass C using a $k_{EC}$ conversion factor of 0.45 (Wu et al. 1990).

### Rhizosphere soil DNA

Measuring rhizosphere DNA content gives an indication of changes in bacterial and fungal abundances according to geocytotype. Nine month old plants were taken from the pots and shaken carefully to remove the nonadhering soil particles. Then a brush was used to gently remove the adhering rhizosphere soil particles from plant roots, which were passed through a 1 mm sieve and stored in a DNA extraction buffer (Frey et al. 2006) at −20°C until analysis. Soil DNA from the rhizosphere samples was prepared by a bead beating procedure (Frey et al. 2008). Briefly, 0.5 g of soil (dry weight) was subjected to three repeated extractions using a bead beater (FP 120; Savant Instruments, Farmingdale, NY, USA). Supernatants from all three extractions were pooled and subsequently purified with a chloroform–isoamyl alcohol (24/1) extraction. DNA was recovered after ethanol precipitation and resuspended in TE buffer, pH 8 (10 mM Tris–HCl, 0.1 mM EDTA, pH 8). DNA concentrations were determined using a fluorometric assay with PicoGreen (Molecular Probes, Eugene, OR, USA). The DNA concentration was adjusted to 10 ng $\mu l^{-1}$ with Tris–EDTA. Before PCR, the soil DNA was pretreated with bovine serum albumin (BSA) to bind humic acids and other PCR-inhibiting substances.

### Amplification of bacterial 16S rRNA gene fragments

Bacterial 16S ribosomal RNA genes were amplified by the PCR using fluorescently labelled (6-FAM) forward primer 27f and unlabeled reverse primer 1378r (Heuer et al. 1997) in a total volume of 50 μl reaction mixture containing 20 ng of template DNA, 1× PCR-buffer (Qiagen, Hilden, Germany), 2 mM $MgCl_2$, 0.2 μM of each primer, 0.4 mM deoxynucleoside triphosphate (Promega, Madison, WI, USA), 0.6 mg $ml^{-1}$ BSA (Fluka, Buchs, Switzerland), and 2 U HotStar *Taq* polymerase (Qiagen). PCR amplification was performed with the following cycling conditions: an initial activating step for HotStar Taq-polymerase (15 min at 95°C), followed by 35 cycles with denaturation for 45 s at 94°C, annealing for 45 s at 48°C, and extension for 2 min at 72°C, with final extension for 5 min at 72°C. The PCR amplification was then completed with an additional final extension step at 72°C for 5 min. Amplified DNA was verified via electrophoresis of aliquots of PCR mixtures (5 μl) on a 1% agarose gel in 1% TAE buffer.

### Terminal restriction enzyme fragment length polymorphism analysis (T-RFLP)

Following confirmation of a successful PCR reaction by agarose gel electrophoresis, the PCR products were purified with the Montage PCR purification clean-up kit (Millipore Corporation, Billerica, MA, USA). Purified PCR products were digested with 2U of the restriction endonuclease *Msp*I or *Hae*III (Promega) and incubated overnight at 37°C. Aliquots (5 μl) of digestion products were verified on a 2% agarose gel in 1% TAE buffer. Prior to the T-RFLP analysis, digests were desalted with Montage SEQ96 sequencing reaction clean-up kit (Millipore Corporation), according to the manufacturer's instructions. T-RFLP analyses were performed according to Frey et al. (2006). Two microlitres of digested PCR products were analysed along with 0.2 μl of internal size standard ROX500 (Applied Biosystems, Foster City, CA, USA) and 12 μl HiDi formamide (Applied Biosystems) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with 36 cm capillaries filled with POP-4 polymer. T-RFLP profiles were analysed using Genotyper v3.7 NT (Applied Biosystems) with a signal threshold of 50 relative fluorescence units. Normalisation of the T-RFLP profiles was performed according to Blackwood et al. (2003). Due to time constraints, analyses of DNA content and T-RFLP were only done on 13 individuals of *C. maculosa* (four native diploids, four native tetraploids and five introduced tetraploids) and 17 individuals of *S. inaequidens* (five native diploids, six native tetraploids and six introduced tetraploids).

We used the Shannon–Weaver index ($H$) to assess bacterial diversity based on TRF peaks using the number and the sizes of the TRF peaks for each profile according to the equation:

$$H = C/N\left(N\log_{10}N - \sum n_i\log_{10}n_i\right) \quad (1)$$

where $C = 2.3$, $N =$ sum of peak heights, $n_i =$ height of TRF $i$, and $i =$ number of TRFs in each T-RFLP profile (Brodie et al. 2003).

### Numerical analyses

All of the analyses were carried out with R2.7.2 (R Development Core Team, 2008). Differences in microbial biomass carbon per gramme of root dry mass, rhizosphere total DNA content and bacterial Shannon–Weaver diversity index according to species and geocytotype were analysed using analysis of variance (ANOVA). To reduce heteroscedasticity, rhizosphere total DNA content was log-transformed. Since effects of polyploidisation or introduction in the new range can be species specific, analyses

were also performed separately for each model species, followed by Tukey post hoc tests.

To study the multivariate response of the T-RFLP profiles, we first used nonmetric multidimensional scaling analyses (NMDS) on a chord distance matrix to look at the distribution of the geocytotype rhizosphere bacterial T-RFLP profiles for each model species. NMDS was chosen since it preserves ordering relationships among objects (Legendre and Legendre 1998) while representing them in a few dimensions. In a second step, a set of redundancy analyses (RDA) constraining the bacterial T-RFLP profile of each model species by geocytotype, ploidy level, range, and range for tetraploid cytotypes was performed. Permutation tests were used to assess the significance of these multivariate regression models.

Lastly, we performed multiple factorial analyses (MFA) to get an overview of the correlations between plant identity, plant traits, rhizosphere characteristics, and rhizosphere bacterial T-RFLP profiles of each model species. We built four data matrices: one matrix contained information about individual identity (geocytotype), while a second matrix contained data on leaf (SLA), vegetative (root biomass, root–shoot ratio), and reproductive (capitulum production, onset of flowering) traits of the individuals growing in the pot. The two last matrices contained information on the individual's rhizosphere characteristics. While one matrix contained results from T-RFLP bacterial profiles, the other one contained quantitative data on rhizosphere microbial biomass carbon, total DNA content and bacterial diversity as expressed by the Shannon–Weaver index. We used only the plant traits and rhizosphere biota matrices, adding plant identity matrix afterwards as passive information. The significance of the correlations between the matrices was tested using Mantel tests. Since we found a correlation between plant traits of *S. inaequidens* and biota characteristics of its rhizosphere, we performed an RDA constraining microbial biomass carbon, DNA content and Shannon–Weaver diversity index by plant traits. The best model was chosen according to forward selection and AIC criteria.

## Results

### Microbial biomass carbon, total DNA content, and bacterial diversity in the rhizosphere

Microbial biomass carbon in the rhizosphere ranged from 273 to 749 mg C $g^{-1}$ roots and was significantly higher in the rhizosphere of *S. inaequidens* than in that of *C. maculosa* (Fig. 1a, $F_{1,39} = 14.857$, $p < 0.001$). We found no significant differences among geocytotypes for either of the two species (Table 1; Fig. 1a). Rhizosphere DNA content ranged from 212 to 377 µg DNA $g^{-1}$ dry soil, and there were no differences between species either (Fig. 1b, $F_{1,28} = 0.031$, $p = 0.862$). There were no differences in rhizosphere DNA content among geocytotypes of *C. maculosa*. By contrast, polyploidisation in the native range of *S. inaequidens* led to a marginally significant decrease in DNA content (Table 1; Fig. 1b). The rhizosphere bacterial Shannon–Weaver diversity index of *Centaurea maculosa* was higher than that of *S. inaequidens* (Fig. 1c, $F_{1,28} = 4.398$, $p = 0.045$). There were no differences among the geocytotypes of *S. inaequidens*, whereas bacterial diversity in the rhizosphere of *C. maculosa* increased following polyploidisation and introduction in the new range (Table 1; Fig. 1c).

### T-RFLP profiling

Rhizosphere bacterial communities differed marginally between the two plant species, as shown by the results for the RDA constraining bacterial profile by species ($F_{1,28} = 2.001$, $p = 0.076$, 999 Monte Carlo permutations, not shown). The differentiation of T-RFLP profiles according to geocytotype was species specific. While T-RFLP profiles of *C. maculosa* were clearly separated among geocytotype along the first dimension of the NMDS (Fig. 2a), the pattern was less clear for *S. inaequidens* (Fig. 2b). This was confirmed by the RDAs constraining the T-RFLP profiles by geocytotype, ploidy or range, which showed no differentiation of bacterial profiles of *S. inaequidens* among geocytotypes. By contrast, bacterial profiles of *C. maculosa* were significantly different according to geocytotype, ploidy and range among tetraploid cytotypes (Table 2). Lastly, the T-RFLP profiles of individuals from the same population were not more similar than profiles of other individual of the same geocytotype, as assessed by their relative positions in the NMDS ordinations (Fig. 2a, b).

### Correlations among plant traits, rhizosphere biota characteristics and plant identity

We did not observe any correlation between bacterial T-RFLP profiles and other rhizosphere biota characteristics for either species (Table 3), indicating that changes in bacterial profiles did not lead to changes in microbial biomass carbon, total DNA content or bacterial diversity. For both species, plant traits were not correlated to geocytotype, suggesting that the traits we measured were not significantly different according to the geocytotype considered. Plant traits of *C. maculosa* were neither correlated to bacterial T-RFLP profiles nor to rhizosphere biota characteristics (Table 3). However, rhizosphere T-RFLP profiles were significantly correlated to geocytotype (Table 3). By contrast, plant traits of *S. inaequidens* were

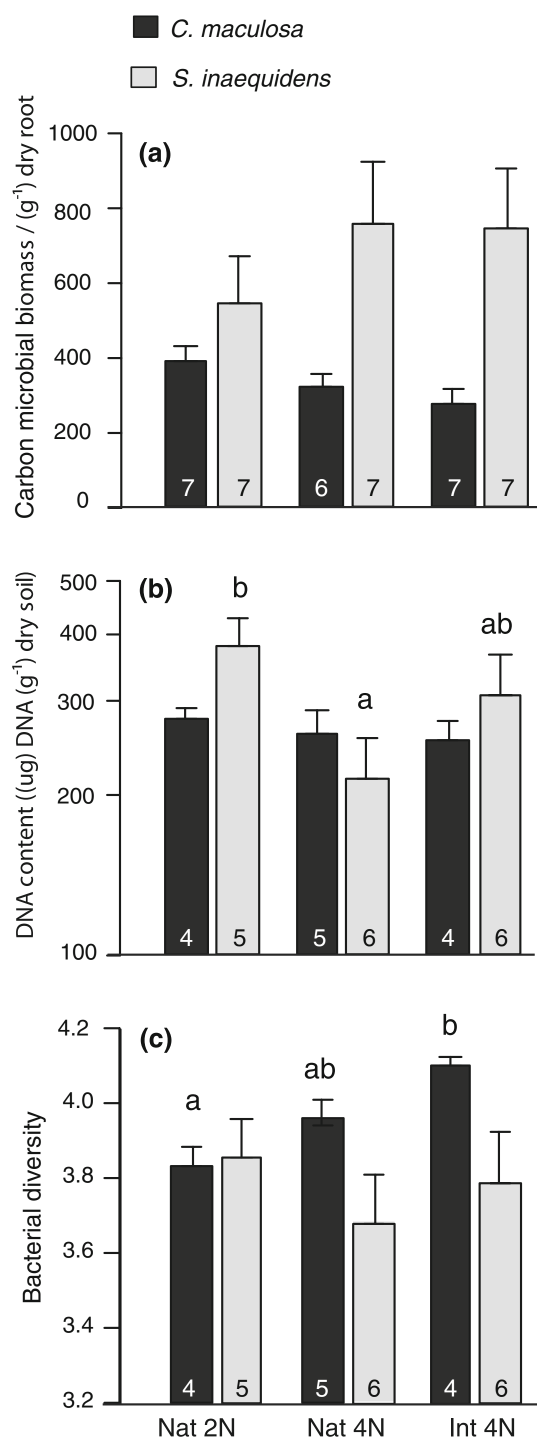


**Fig. 1** **a** Microbial biomass carbon per unit roots; **b** total DNA content, and; **c** bacterial diversity in the rhizospheres of the geocytotypes ("Nat 2*N*", native diploid; "Nat 4*N*", native tetraploid; "Int 4*N*", introduced tetraploid) of *C. maculosa* (*black*) and *S. inaequidens* (*grey*). Sample sizes are indicated at the *bottom* of each histogram. *Bars* are means with standard errors. DNA content is represented on a logarithmic scale. *Letters* represent differences between geocytotypes according to Tukey post hoc tests ($p < 0.10$)

significantly correlated to rhizosphere biota characteristics such as microbial carbon biomass, total DNA content or bacterial diversity, but there was no significant correlation between geocytotype and rhizosphere T-RFLP profile (Table 3). In the RDA constraining the rhizosphere biota characteristics of *S. inaequidens* by plant traits, plant traits explained 48.1% of the variance ($F_{4,12} = 2.785$, $p = 0.009$, 999 Monte Carlo permutations). In the RDA biplot, bacterial diversity and DNA content of the rhizosphere were associated with late flowering and root biomass. By contrast, microbial biomass carbon in the rhizosphere was negatively correlated with capitulum production of *S. inaequidens* (Fig. 3).

## Discussion

We did not observe any difference in rhizosphere microbial biomass carbon among geocytotypes of *C. maculosa* and *S. inaequidens*. This shows that polyploidisation does not affect overall rhizosphere microbial biomass carbon, and that introduced genotypes do not inhibit overall microbial growth as compared to native ones for these two species. However, microbial biomass does not give information on potential effects on the structure of bacterial communities.

In agreement with our hypothesis, we observed effects of polyploidisation on bacterial community composition. However, these effects were species specific. Indeed, polyploidisation led to a marginal decrease in the rhizosphere DNA content of *S. inaequidens*, while in the case of *C. maculosa*, it led to an increase in bacterial diversity and changes in bacterial T-RFLP profiles. This suggests that, while polyploidisation affected mainly rhizosphere bacterial communities of *C. maculosa*, other microbes (e.g. fungi, protists) may have been affected in the case of *S. inaequidens* as DNA content decreased but bacterial communities did not change. Furthermore, for both species, DNA content, Shannon–Weaver diversity, and bacterial profiles of individuals within populations were as distinct as between populations (not shown). Therefore, we can argue that contribution of seed-borne organisms was not significant enough to intervene as a confounding effect.

As an evolutionary process, polyploidisation can lead to changes in trait trade-offs (Blossey and Notzold 1995; Bossdorf et al. 2004) or plant chemistry (Dhawan and Lavania 1996; De Jesus-Gonzalez and Weathers 2003; Kim et al. 2004; Hull-Sanders et al. 2009). Polyploidisation could induce quantitative or qualitative changes in the production of secondary compounds. Since these compounds may affect microbial and fungal communities (Hol

**Table 1** Analysis of variance on microbial biomass carbon, DNA content and rhizosphere bacterial diversity (Shannon–Weaver diversity index) for *C. maculosa* and *S. inaequidens*

| | | Microbial biomass C per unit root | | | Rhizosphere total DNA content | | Bacterial diversity (Shannon index) | |
|---|---|---|---|---|---|---|---|---|
| | *d.f.* | *F* value | *P* value | *d.f.* | *F* value | *P* value | *F* value | *P* value |
| *Centaurea maculosa* | | | | | | | | |
| Geocytotype | 2 | 2.05 | 0.16 | 2 | 0.37 | 0.70 | 5.82 | 0.02 |
| Residuals | 17 | | | 10 | | | | |
| *Senecio inaequidens* | | | | | | | | |
| Geocytotype | 2 | 0.60 | 0.56 | 2 | 2.98 | 0.08 | 0.50 | 0.62 |
| Residuals | 18 | | | 14 | | | | |

DNA content was log-transformed prior to the analyses

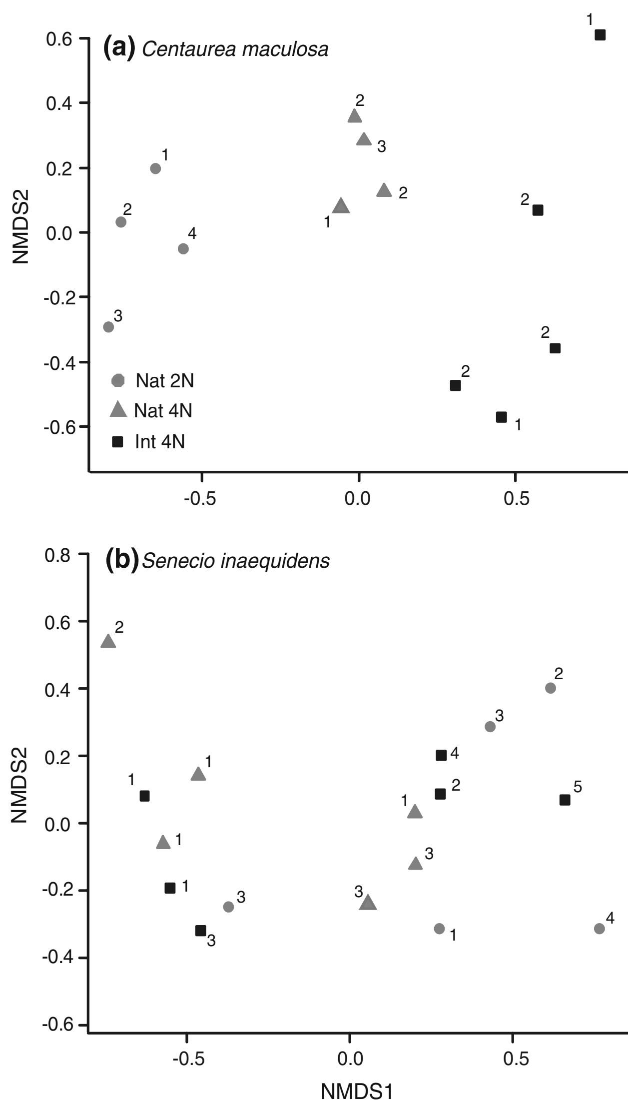


**Fig. 2** Nonmetric multidimensional scaling analysis of T-RFLP profiles of native diploid ("Nat 2*N*"), native tetraploid ("Nat 4*N*") and introduced tetraploid ("Int 4*N*") geocytotypes of **a** *C. maculosa* and **b** *S. inaequidens*. Stress values are respectively 3.74 and 10.94. *Numbers* (*1–5*) near symbols represent the population (as designed in Table 1 of the "ESM"). For each geocytotype, individuals with the same number come from the same population

**Table 2** Results of Monte Carlo permutation tests (999 permutations) following redundancy analysis done on T-RFLP profiles of each species, testing one factor each time: geocytotypes (native 2*N*, native 4*N*, introduced 4*N*), ploidy (2*N* vs. 4*N*), range (native vs. introduced) and range for tetraploid cytotypes (native 4*N* vs. introduced 4*N*)

| Factor | Variance explained (%) | *F* value | *P* value |
|---|---|---|---|
| *Centaurea maculosa* ($n = 13$) | | | |
| Geocytotype | 57.54 | $F_{2,10} = 6.78$ | <0.01 |
| Ploidy | 43.28 | $F_{1,11} = 8.39$ | <0.01 |
| Range | 9.71 | $F_{1,11} = 1.18$ | 0.27 |
| Range (4*n*) | 33.59 | $F_{1,7} = 3.54$ | <0.01 |
| *Senecio inaequidens* ($n = 17$) | | | |
| Geocytotype | 14.96 | $F_{2,14} = 1.23$ | 0.24 |
| Ploidy | 9.65 | $F_{1,15} = 1.60$ | 0.12 |
| Range | 4.40 | $F_{1,15} = 0.69$ | 0.65 |
| Range (4*n*) | 7.59 | $F_{1,10} = 0.82$ | 0.55 |

and Van Veen 2002; Kowalchuk et al. 2006), it can be hypothesised that polyploidisation could affect plant–soil interactions so as to give a competitive advantage to invasive species through modification of the soil biota. Changes in rhizosphere bacterial communities may be the result of increased production or qualitative changes of secondary metabolites. Testing this hypothesis would require precise assessments of the volume, composition, and concentration of leachates (either from root exudates or tissue decomposition) for each geocytotype, which was beyond the scope of this study.

In accordance with our hypothesis, we observed species-specific responses of bacterial T-RFLP profiles (Fig. 2a, b), which highlights the difficulties involved in elucidating a general pattern. Bacterial community structure did not vary among geocytotypes for *S. inaequidens*. By contrast, rhizosphere bacterial profiles of *C. maculosa* differed according to geocytotype, leading to three distinct bacterial communities. Among tetraploid individuals of *C. maculosa*, bacterial communities also differed between seeds from the native range and seeds from the introduced one,

**Table 3** Table of correlations between matrices used in the multiple factorial analyses of *C. maculosa* and *S. inaequidens*

| | T-RFLP profiles | Rhizosphere characteristics | Plant traits | Geocytotype |
|---|---|---|---|---|
| *Centaurea maculosa* | | | | |
| T-RFLP profiles | 1.000 | | | |
| Rhizosphere characteristics | 0.110 | 1.000 | | |
| Plant traits | 0.140 | 0.023 | 1.000 | |
| Geocytotype | 0.754*** | 0.190 | 0.059 | 1.000 |
| *Senecio inaequidens* | | | | |
| T-RFLP profiles | 1.000 | | | |
| Rhizosphere characteristics | 0.268 | 1.000 | | |
| Plant traits | 0.146 | 0.297* | 1.000 | |
| Geocytotype | 0.223 | 0.127 | 0.054 | 1.000 |

The significance of a correlation is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

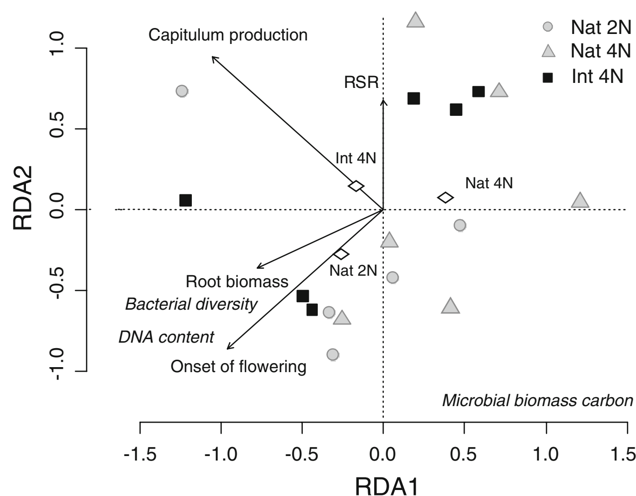


**Fig. 3** Redundancy analysis biplot of the rhizosphere biota characteristics of *S. inaequidens* (*in bold*) constrained by plant traits (*arrows*). *Symbols* represent native diploid ("Nat 2*N*"), native tetraploid ("Nat 4*N*") and introduced tetraploid ("Int 4*N*") geocytotypes. *Diamonds* represent centroids of geocytotypes. *Axes* 1 and 2 hold, respectively, 35.4% ($F_{1,12} = 8.19$, $p = 0.02$) and 12.0% ($F_{1,12} = 2.79$, $p = 0.34$) of the explained variance

which suggests that a belowground mechanism may be involved in the invasive success of this species. The invasion success of *C. maculosa* has long been thought to be due to the release of an allelopathic chemical into the soil (Ridenour and Callaway 2001; Callaway and Ridenour 2004), but opinions vary on this question (Blair et al. 2006; Perry et al. 2007). Our findings suggest a possible invasion mechanism related to the ability to modify bacterial communities. This mechanism would agree with the theory of accumulation of local pathogens, according to which plants are able to accumulate pathogens in their rhizospheres by amplifying a subset of the bacterial communities (Eppinga et al. 2006; Mangla et al. 2008). The accumulation of pathogens is thought to be more noxious for neighbouring plants than for the exotic plant itself, which gives it a competitive advantage in the community.

The bacterial communities of *Senecio inaequidens* do not seem to change significantly according to geocytotype. However, although bacteria are likely to be first affected by changes in the quantity or quality of root exudates, other soil biota and/or soil physical properties might have been affected by polyploidisation, as suggested by the decrease in the total DNA content of the rhizosphere.

To our knowledge, this study is the first attempt in the framework of biological invasion to link plant traits with their rhizosphere bacterial communities, and polyploidisation. Assessing correlations between plant traits and rhizosphere biota characteristics can provide insight into the pathway involved in invasion success, taking into account plant attributes. We did not find any significant correlations between plant traits and changes in bacterial community profiles of the rhizosphere, leading to the conclusion that the subset of traits we studied does not affect rhizosphere bacterial community structure. Nevertheless, plant traits were significantly correlated with other rhizosphere biota characteristics (total DNA content, bacterial diversity, and microbial biomass carbon) in the case of *S. inaequidens*, which illustrates the importance of interspecies differences.

Root biomass and reproductive output of *S. inaequidens* were correlated with changes in their soil rhizosphere biota. By contrast, variations in individuals' SLA did not influence soil rhizosphere biota (SLA was not selected in the best model of RDA), indicating no effect of nutrient cycling strategy or leaf properties on these characteristics of the belowground compartment. Late flowering and plant root biomass were positively correlated with Shannon–Weaver bacterial diversity index and DNA content. It appears that preferential allocation of resources to belowground tissues, at the expense of reproductive effort, may favour the development of rhizosphere communities, either in bacteria or in other functional groups in the early developmental stages of *S. inaequidens*.

Our results call for more attention to be paid to the potential effects of polyploidisation and plant functional traits on rhizosphere biota. Studying belowground mechanisms and interactions between aboveground and belowground compartments could lead to a better understanding of invasion success. Since polyploidisation is recognised as a common attribute of invasive species, research linking evolutionary changes to rhizosphere characteristics should be given high priority. This study highlights the difficulties involved in elucidating general patterns of invasion

mechanisms, since even taxonomically related invasive species tend to present different invasion mechanisms.

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