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Effects of invasive aquatic carrion on soil chemistry and terrestrial microbial communities

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Abstract Carrion plays a crucial role in the recycling of nutrients and organic matter in ecosystems. Yet, despite their ecological importance, studies addressing the relevance of carrion originated from invasive alien species (IAS) in the interface between aquatic and terrestrial ecosystems are uncommon, especially those assessing belowground effects. In this study, we carried out a manipulative experiment to assess the impact of massive mortalities of the Asian clam *Corbicula fluminea* (Müller, 1774) as a carrion subsidy evaluating possible effects on the terrestrial soil chemistry and the structure of a microbial (bacteria and fungi) community. We placed five levels of *C. fluminea* density (0, 100, 500, 1000 and 2000 ind. m^{-2}) and samples were collected 7, 30 and 90 days after clams' addition. The results revealed that *C. fluminea* carrion have a significant effect belowground, especially on nutrients content (mainly NH_4^+, NO_2^-, NO_3^- and PO_4^{3-}), fungal biomass and fungal and bacterial diversity. Given the predicted increase and intensification of extreme climatic events and the widespread distribution of several aquatic IAS (including bivalve species such as *C. fluminea*) the ecological importance of these massive mortalities (and resulting carrion) cannot be ignored because they may affect microbial communities with significant impacts on nutrient cycling, even in adjacent terrestrial habitats.

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Introduction

Decomposition of detritus plays a crucial role in the recycling of nutrients and organic matter (Swift et al. 1979; Moore et al. 2004). Defined as any resource of nonliving organic matter, detritus is considered the basal trophic level of many terrestrial and aquatic food webs (Swift et al. 1979; Moore et al. 2004; Benbow et al. 2015). In terrestrial ecosystems, detritus may consist of plant-derived matter (e.g. leaf litter, dead

wood, root exudates), dead microbes, fecal matter and animal tissue (carrion) (Swift et al. 1979). Plant-derived detritus comprises the majority of the resources that undergo decomposition in terrestrial ecosystems but they are nutrient poor and very recalcitrant (Swift et al. 1979; Carter et al. 2007). Contrary, carrion is much more nutrient-rich and decomposes at much faster rates than plant detritus, and, as such, its role in nutrient cycling may be highly relevant (Swift et al. 1979; Barton et al. 2013). Indeed, carrion decomposition is usually associated with the activity of microbes, invertebrate and vertebrate detritivores and scavengers (Carter et al. 2007; Barton et al. 2013). The combined activity of microbes and invertebrates increases the nutrients released from the carrion into the soil; however, vertebrates may reduce this contribution by direct consumption or dispersion (Carter et al. 2007; Barton et al. 2013). In terrestrial ecosystems where carrion releases nutrients into the soil, plants may ultimately mobilize these nutrients entering the belowground detrital pathway (Moore et al. 2004; Carter et al. 2007). Despite its potential importance, only very recently the scientific community has started to acknowledge the role of carrion decomposition in nutrient recycling (DeVault et al. 2003; Carter et al. 2007; Wilson and Wolkovich 2011).

Detrital inputs enter any ecosystem via allochthonous or autochthonous sources (Moore et al. 2004). Allochthonous inputs are resources that originate in one habitat but move into another, while autochthonous inputs originate and are consumed in the same habitat (Polis et al. 1997). Many ecosystems experience spatial subsidies as regular seasonal events, but subsidies can also result from sporadic episodes of resource superabundance, named resource pulses (Ostfeld and Keesing 2000; Anderson et al. 2008b; Yang et al. 2008). Examples of resource pulses include periodical cicadas' emergence in North American forests (e.g. Yang 2004, 2008), El Niño rainfalls in arid ecosystems (e.g. Polis et al. 1997; Meserve et al. 2003; Letnic et al. 2005), seed or fruit mast events (e.g. Woff 1996; Curran and Leighton 2000), and massive spawning events by migratory fish (e.g. Woff and Hershey 1999; Yanai and Kochi 2005). Despite their ecological importance, these studies focused mostly on aboveground processes and just a few assessed belowground effects (Yang 2004; Yang et al. 2010). For example, Yang (2004) investigated the belowground effects of cicada massive

mortalities and reported increases on nitrogen availability and microbial biomass in forest soils and on plants growth and reproduction.

Terrestrial and freshwater ecosystems can receive both autochthonous and allochthonous subsidies, although autochthonous inputs are more common in terrestrial ecosystems and allochthonous inputs in freshwater ecosystems (Nowlin et al. 2008). Due to the geographic position of freshwater ecosystems in the landscape, usually the allochthonous inputs are in the land–water direction (Shurin et al. 2006). However, some studies have verified that freshwater ecosystems can also transfer resources to the adjacent terrestrial ecosystems. Aquatic insect emergence (Henschel et al. 2001; Sabo and Power 2002), lateral spread of nutrients by large herbivores (Bump et al. 2009a; Doughty et al. 2013), migrations of fish (Moore et al. 2007) and extreme riverine flood pulses (Junk et al. 1989; Sousa et al. 2012) are some examples. In addition, and since freshwater ecosystems are subject to numerous introductions of invasive alien species (IAS), allochthonous inputs in the water-land direction mediated by IAS may also occur (Bódis et al. 2014). Nevertheless, few studies report these phenomena and fewer assess their possible ecological impacts (Sousa et al. 2014). Recently, Novais et al. (2015) found that the biomass resulting from massive mortalities of the Asian clam *Corbicula fluminea* (Müller, 1774) functions as a resource pulse to aboveground consumers, namely terrestrial invertebrates. Interestingly, abundance, biomass, richness and diversity of terrestrial invertebrates responded positively to *C. fluminea* carrion addition and clear temporal differences were also detected (Novais et al. 2015). Similarly to the aboveground effects, *C. fluminea* carrion may also result in significant belowground effects with possible changes on soil chemistry and terrestrial microbial communities.

Given the limited understanding of carrion in belowground processes, mainly carrion derived from IAS, we carried out a manipulative experiment under natural conditions simulating a *C. fluminea* mortality event. It is important to mention that this experiment is part of a larger study that aimed at understanding the impact of massive mortalities of *C. fluminea* as a resource pulse to terrestrial communities. The first part of the study assessed possible effects on a terrestrial invertebrate community (Novais et al. 2015). Here we assessed possible effects on soil chemistry and on the

structure of a terrestrial microbial community. We hypothesized that *C. fluminea* carrion would increase nutrients content and the biomass and diversity of a microbial (fungi and bacteria) community; however, this increase would be time dependent.

Materials and methods

Study area and experimental setup

The Minho River (NW of Iberian Peninsula) was selected to carry out this experiment since in recent years massive mortalities of *C. fluminea* after extreme climatic events have been reported (see Ilarri et al. 2011; Sousa et al. 2012). The selected area is 40 km upstream the river mouth and approximately 250 m inland in the left river bank (42° 04′ 28.12″N, 08° 31′ 29.14″W), characterized by sandy soils with low organic matter. A more detailed description of the selected area and soil characterization can be found in Ilarri et al. (2015). Although relatively further inland, earlier data (Ilarri et al. 2015) confirm the reliability of this site in reproducing the magnitude of massive mortalities of *C. fluminea* after a great flood. Indeed, during the 2001 flood a great accumulation of dead *C. fluminea* [average (± SD) density values of 2367.5 ± 1023.90 ind. m^{-2}] was reported in the studied area (Ilarri et al. 2015).

In the last decades, several IAS were introduced in the downstream area of the Minho River with *C. fluminea* being especially problematic (for details see Sousa et al. 2005, 2007, 2008a, b; Costa-Dias et al. 2010; Mota et al. 2014). Currently, the presence of *C. fluminea* dominates the benthic community, contributing with more than 95% to the total benthic biomass in the Minho River international section (Sousa et al. 2008a, c). During the winters of 2000/2001 and 2009/2010 major floods occurred in the Minho River and a substantial quantity of bivalves (*C. fluminea* and other native species such as *Potomida littoralis* (Cuvier, 1798), *Unio delphinus* (Spengler, 1793) and *Anodonta anatina* (Linnaeus, 1758); for details see Sousa et al. 2012; Ilarri et al. 2015) was moved to the adjacent river banks. These bivalves suffer massive mortalities when water levels return to normal at the end of spring/beginning of summer. For example,

during the major floods of 2009/2010, Sousa et al. (2012) reported mean density and biomass values of dead bivalves of 1043 ind. m^{-2} and 5726 g wet weight. m^{-2}, respectively, along five sites on the left bank of the Minho River, where *C. fluminea* represented approximately 99% of the total biomass found.

In order to assess the possible effects of the massive mortalities of *C. fluminea* on soil chemistry and on the terrestrial microbial community a manipulative field experiment was conducted. *C. fluminea* individuals were collected 48 h before the experiment, frozen and posteriorly used in the experiment using a randomized complete block design with three blocks. Each block contained five 1 m^2 plots corresponding to five levels of manipulated *C. fluminea* density: 0, 100, 500, 1000 and 2000 ind. m^{-2}. These levels were selected to mimic a range of *C. fluminea* densities observed when massive mortalities occur resulting from major floods (Sousa et al. 2012; Ilarri et al. 2015). In order to minimize inter-plot interactions and habitat variability, plots were distributed within a grid of c.a. 1 m interval. The experiment lasted 3 months (June–September 2013) and samples were collected 7, 30 and 90 days after *C. fluminea* addition. A more detailed description of the experimental design can be found in Novais et al. (2015).

Surface soil samples (1 cm depth) were collected with a small core (3–5 cm^2) for nutrient assessment, fungal biomass quantification and analysis of microbial (fungal and bacterial) diversity. Multiple surface soil samples in each plot were randomly collected, homogenized (mixed and a small sub-sample randomly taken) and deep frozen at −80 °C.

Soil chemistry characterization

Concentrations of organic carbon (C), total nitrogen (N), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), calcium (Ca) and potassium (K) were measured in Centro de Apoio Científico e Tecolóxico á Investigación (CACTI), University of Vigo, Vigo (Spain) following standard procedures. Concentrations of organic C and total N were quantified by dry combustion using a LECO CN 2000. Concentrations of NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} were quantified by standard colorimetric methods using a Bran Lubbe continuous flow auto analyzer (Brand Luebbe AA3) after an extraction in KCl.

Finally, concentrations of Ca and K were quantified by inductively coupled plasma-atomic emission spectrometry (ICP-OES Optima 4300).

Terrestrial microbial community characterization

Fungal biomass was estimated from 1.5 g of soil from each replicate by ergosterol quantification, following Pascoal and Cássio (2004). Lipids extraction was performed by heating (30 min at 80 °C) the sample in 10 mL of 0.8% KOH–methanol and the resulting extract was partially purified by solid-phase extraction (Sep-Pak cartridges, Waters, Milford, MA, USA). Ergosterol was quantified by high-performance liquid chromatography (Beckmann Gold System, Brea, CA, USA) using a LiChrospher RP18 column (250 × 4 mm, Merck), where the system ran isocratically with methanol as mobile phase (1.4 mL min^{-1}, 33 °C). Ergosterol was detected at 282 nm and its concentration was estimated based on a standard curve of ergosterol (Sigma) in isopropanol.

For microbial diversity assessment, DNA was extracted from 200 mg of soil using a DNA extraction kit (PowerSoil DNA Isolation Kit, MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. The ITS2 region of fungal rDNA and the V3 region of bacterial 16S rDNA were amplified with the primer pairs ITS3GC/ITS4 and 338F_GC/518R, respectively (following Duarte et al. 2010).

For polymerase chain reaction (PCR) 2x of Dream GoTaq$^®$ Green Master Mix (Promega), 0.4 μM of each primer and 1 μL of DNA were used in a final volume of 25 μL. A MyCycler Thermal Cycler (BioRad Laboratories, Hercules, CA, USA) was initially used for amplification with a denaturation for 2 min at 95 °C, 36 cycles of denaturation for 30 s at 95 °C, primer annealing for 30 s at 55 °C and extension for 1 min at 72 °C, and a final extension for 5 min at 72 °C (following Duarte et al. 2010).

Denaturing gradient gel electrophoresis (DGGE) analysis was performed using a DCodeTM Universal Mutation Detection System (BioRad Laboratories, Hercules, CA, USA). For fungi and bacteria, samples of 700 ng from the amplified DNA products with 380-400 bp (ITS3GC/ITS4) and 200 bp (338F_GC/518R), respectively, was loaded on 8%

(w/v) polyacrylamide gel in 1x Tris–acetate-EDTA (TAE) with a denaturing gradient from 30 to 70% (100% denaturant corresponds to 40% formamide and 7 M urea). All gels were run at 55 V, 56 °C for 16 h and then were stained with 1 × of GelStar (Lonza) for 10 min. Gel images were captured under UV light using a ChemiDoc XRS (BioRad).

Data analyses

Two-way permutational multivariate analysis of variance (PERMANOVA) (type-III) were used in a two-way crossed designed to test for fixed effects of *C. fluminea* density (five levels: 0, 100, 500, 1000 and 2000 ind. m^{-2}) and time (three levels: 7, 30 and 90 days) on fungal biomass and nutrients content. Variables were standardized without transformation prior to PERMANOVA analyses, with the exception of NO_3^- and PO_4^{3-} that were log (X + 1) transformed. Similarity matrices were also calculated using Euclidean distances (Clarke and Warwick 2001).

For each group of microbes, DGGE gels were aligned and the relative intensity of the band was analyzed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Each DGGE band was considered one operational taxonomic unit (OTU). Differences in the structure of microbial (fungal and bacterial) community were tested by non-metric multidimensional scaling (NMDS) followed by the two-way PERMANOVAs (type-III), with the same design as described above. Variables were standardized without transformation prior to NMDS ordination analyses and similarity matrices were calculated using the Bray Curtis similarity index (Clarke and Warwick 2001).

In PERMANOVA tests, the statistical significance of variance (α = 0.05) was tested using 9999 permutations of residuals within a reduced model. When the number of permutations was <150, the Monte Carlo *p* value was considered. PERMDISP was used in all data to test the homogeneity of multivariate dispersions.

PRIMER analytical software (v.6.1.6, PRIMER-E) with the PERMANOVA + 1.0.1 add-on (Anderson 2001; Anderson et al. 2008a) was used for all statistical tests described above.

Results

Soil chemistry characterization

The results for organic C and total N (%), NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, Ca and K (mg kg^{-1}) in the soil are shown in Fig. 1 and Table S1. Concentrations of NH_4^+ and NO_3^- differed significantly between C. fluminea density (Pseudo-F = 15.51, p = 0.01 and Pseudo-F = 4.99, p = 0.01, respectively) and time (Pseudo-F = 6.31, p = 0.01 and Pseudo-F = 42.87, p = 0.01, respectively) (Fig. 1c, e and Table S1). Carbon and NO_2^- differed significantly only between time (Pseudo-F = 3.95, p = 0.03 and Pseudo-F = 4.74, p = 0.01, respectively), and PO_4^{3-} only between C. fluminea density (Pseudo-F = 5.66, p = 0.01) (Fig. 1a, d, f and Table S1). No significant differences in N, Ca and K were detected among the C. fluminea density and time (Fig. 1b, g, h and Table S1).

Terrestrial microbial community

Fungal Biomass

The mean [± standard error of the mean (SEM)] ergosterol concentration was highest at C. fluminea density of 2000 ind. m^{-2} (14.78 ± 4.65 µg g^{-1} soil) at day 7, and lowest at C. fluminea density of 1000 ind. m^{-2} at day 90 (2.08 ± 0.91 µg g^{-1} soil) (Fig. 2). Ergosterol concentration differed significantly only between C. fluminea density (Pseudo-F = 3.10, p = 0.02).

Microbial diversity

Fungal taxon richness based on DGGE OTUs varied from 13 to 23 OTUs and showed a tendency to increase with C. fluminea densities and time (not shown). The NMDS ordination based on the fungal community is shown in Fig. 3a and significant differences in C. fluminea density (Pseudo-F = 1.92, p = 0.01) and time (Pseudo-F = 2.92, p = 0.01) were detected.

Bacterial taxon richness based on DGGE OTUs varied from 7 to 15 OTUs and had a tendency to increase with C. fluminea densities and time (not shown). The NMDS ordination based on the bacterial community is shown in Fig. 3b and significant differences in C. fluminea density (Pseudo-F = 3.08, p = 0.01) and time (Pseudo-F = 3.37, p = 0.01) were detected.

Discussion

Carrion is a higher quality resource that can have significant effects on soil properties (e.g. nutrients content) and biological communities (Carter et al. 2007; Barton et al. 2013). Nutrients entering the soil through the releases of fluids and transfer of carrion tissues are posteriorly recycled by belowground microbial decomposers (Barton et al. 2013). In the particular case of C. fluminea, the carrion resulting from massive mortalities can release some nutrients into the soil via leaching of the shells and decomposition of the soft tissues, also having a significant effect on fungal biomass and diversity of microbial communities.

Effects on soil chemistry

C. fluminea shells are predominantly made of calcium carbonate ($CaCO_3$) in the crystal form of aragonite (Spann et al. 2010) but also contain trace amounts of many other chemical elements such as Na, Mg, Al, P, S, Cl, and K (Eyster 1986). Although we expected that Ca and K would differ significantly between C. fluminea density and time, no differences were detected (Fig. 1g, h). Considering that the manipulative experiment took place during the summer, which is often characterized by high temperatures and low precipitation in the study area, it is possible that the shells demineralization/leaching process was not enough for these nutrients to accumulate in the soil. In addition, the 3 months' duration of the experiment was probably insufficient to detect these differences.

Nitrogen is present in the environment in a wide variety of chemical forms and it is one of the main constituents of many biopolymers, such as amino and nucleic acids of living organisms (Cammack et al. 2015). Thus, during the decomposition of C. fluminea carrion, we expected the release of inorganic N into the soil. Although the percentage of total N did not differ between C. fluminea density and time, significant differences in the N chemical forms, such as NH_4^+, NO_2^- and NO_3^- were detected (Fig. 1b–e).
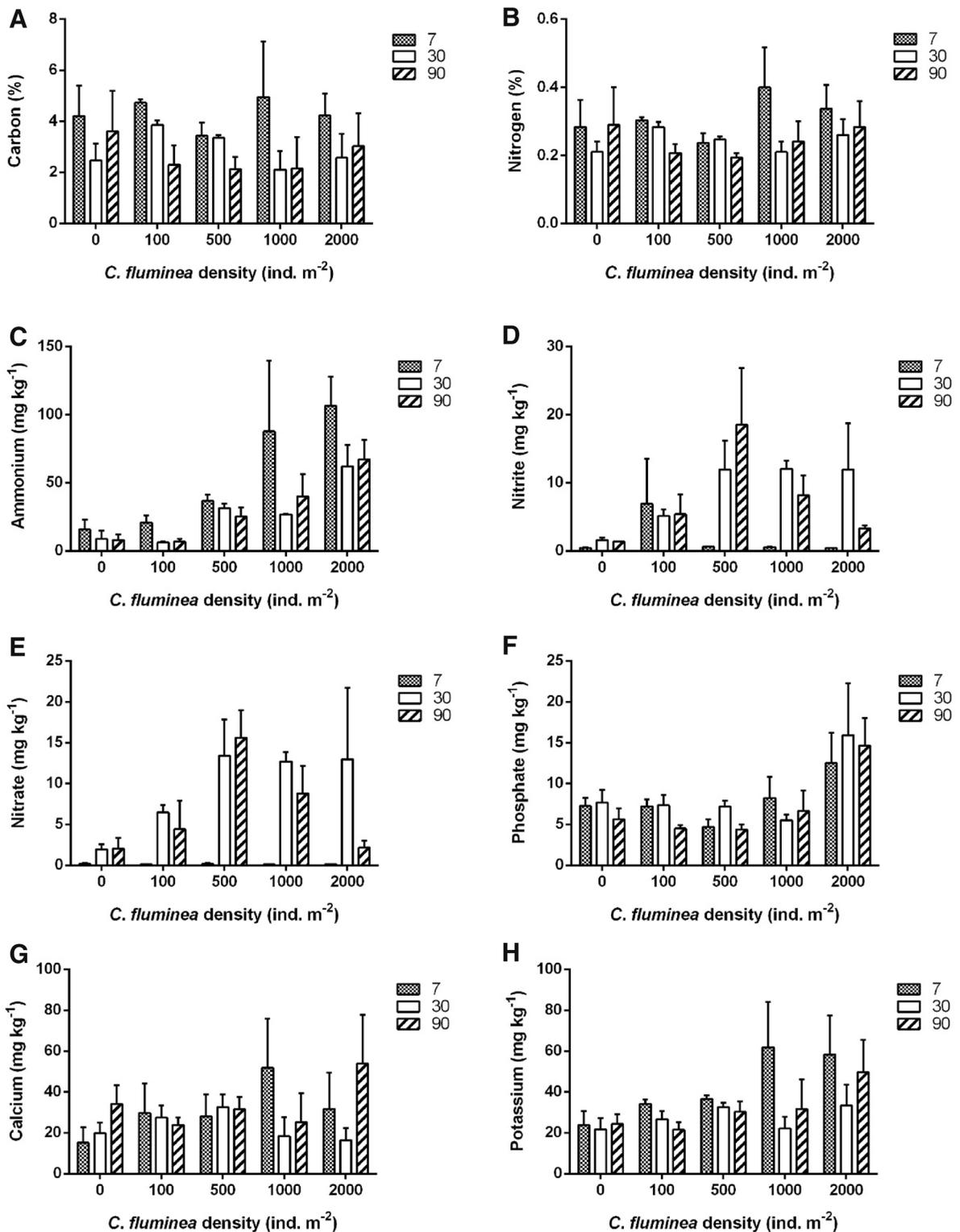
Fig. 1 Mean (±SEM) values of organic C (**a**), total N (**b**) (%), NH_4^+ (**c**), NO_2^- (**d**), NO_3^- (**e**), PO_4^{3-} (**f**), Ca (**g**) and K (**h**) (mg kg^{-1}) at different densities of *C. fluminea* (0, 100, 500, 1000 and 2000 ind. m^{-2}) and sampling times (7, 30 and 90 days)
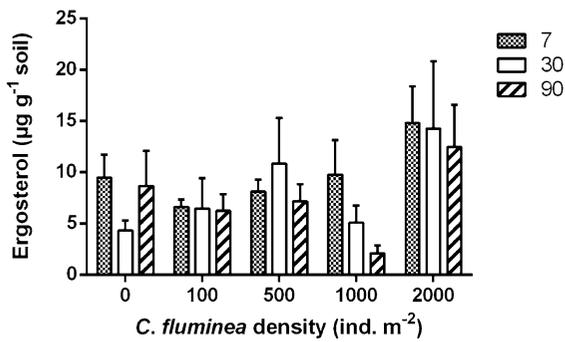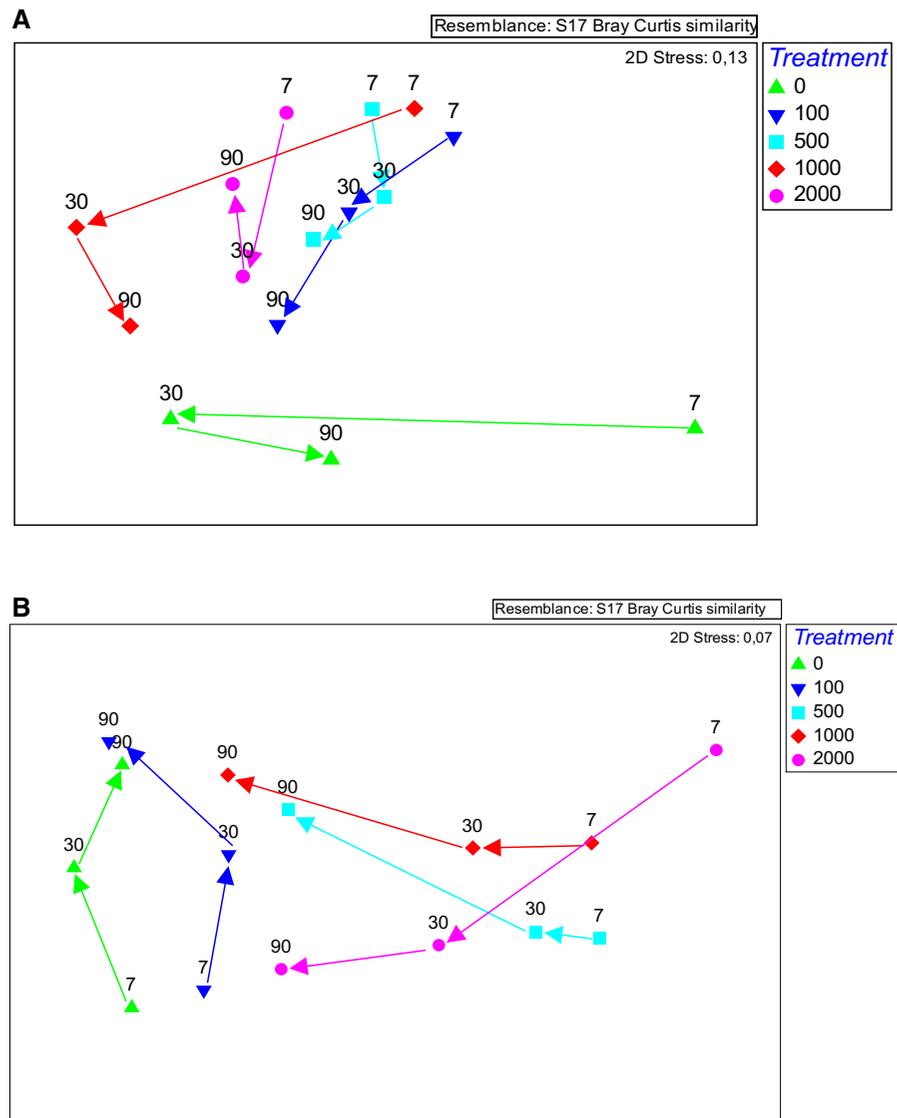
Fig. 2 Mean (±SEM) values of ergosterol concentration ($\mu g \ g^{-1}$ soil) at different densities of *C. fluminea* (0, 100, 500, 1000 and 2000 ind. m^{-2}) and sampling times (7, 30 and 90 days)

The NH_4^+ content significantly responded to *C. fluminea* addition as the highest values were observed in densities higher than 500 ind. m^{-2} and variations over time were also detected as the highest value was obtained at day 7 and declined at days 30 and 90 (Fig. 1c). In contrast, the values of NO_2^- and NO_3^- were low at day 7 independently of *C. fluminea* density, with the exception of 100 ind. m^{-2} in NO_2^-. At day 30, both NO_2^- and NO_3^- values progressively increased mainly for the treatments containing densities of *C. fluminea* higher than 100 ind. m^{-2}. Lastly, at day 90, the values of NO_2^- and NO_3^- remained high for the density of 500 ind. m^{-2}, but progressively decreased in the next two higher *C. fluminea* densities (Fig. 1d, e). These temporal differences in the concentrations of NH_4^+, NO_2^- and NO_3^- seems to be related to the natural process of N cycling, which transforms N from one form to another. When an animal dies, the organic N available in the soil is converted into NH_4^+ by fungi and both aerobic and anaerobic bacteria (Bothe et al. 2007). Thereafter, the nitrification occurs through nitrifying bacteria in two stages: first, the NH_4^+ is converted into NO_2^- and second, NO_2^- is oxidized into NO_3^-. Indeed, pulses of nitrogen-rich detritus result in a momentary acceleration of nitrogen mineralization (Wardle 2002). Similarly to our manipulative experiment, Yang (2004) observed temporal variations in NH_4^+ content. In that study, and using carcasses of cicadas, soil NH_4^+ significantly increased in treatment plots with 240 cicadas m^{-2} when compared to control in the first 30 days of the experiment, while in the subsequent 70 days no effects were detected. In the case of NO_3^-,

the effects were more persistent over time, the NO_3^- availability significantly increased relatively to control during the first 30 days and this pattern was prolonged for the 70 subsequent days (Yang 2004).

Other examples in literature also showed increased content of NH_4^+ and NO_3^- in the soil around animal carcasses (see for example Hopkins et al. 2000; Towne 2000; Bump et al. 2009b; Parmenter and MacMahon 2009). However, in most studies, measurements were performed one or more years after carcasses addition, making the comparison with our results challenging (Hopkins et al. 2000; Towne 2000; Parmenter and MacMahon 2009). For example, Bump et al. (2009b) placed several ungulate carcasses in a North American hardwood forest and found that the content of NH_4^+ and NO_3^- (but not of P, K, Mg and Ca) in soil significantly increased after 3 months. Interestingly, the NH_4^+ values observed in our results for *C. fluminea* density of 2000 ind. m^{-2} at day 90 were significantly higher than the control and were similar to those obtained by Bump et al. (2009b) (67.27 ± 14.14 and $\approx 46 \ mg \ kg^{-1}$, respectively). Hence, our experiment suggests that the decomposition of *C. fluminea* carrion releases nitrogenous compounds into the soil, which had an effect on N cycle and consequently on NH_4^+, NO_2^- and NO_3^- availability.

Our manipulative experiment showed that significant differences in C were only detected over time with values at day 7 higher than those observed on days 30 and 90 (Fig. 1a). Carcasses may release a significant pulse of C into to the soil during decomposition, but this pulse results in a localized microbial activity, which in turn rapidly mineralized organic C into CO_2 (Carter et al. 2007). Indeed, soils with animal carcasses contain more C compared to control but also had higher CO_2 (Hopkins et al. 2000; Carter and Tibbett 2006). According to Carter and Tibbett (2006), a soil incubated at 12 and 22 °C caused an immediate release of CO_2 that peaked on day 2. Unfortunately, we did not measure CO_2 during our experiment and despite the absence of significant differences our results seem to follow the sequence of events described above: first, as a consequence of the decomposition process, C content increased in the soil, and, subsequently, organic C was possibly mineralized and released into the atmosphere contributing to decreased C content in the soil over time.

Fig. 3 Non-metric multidimensional scaling (NMDS) plot of fungal (**a**) and bacterial (**b**) community at different densities of *C. fluminea* (0, 100, 500, 1000 and 2000 ind. m^{-2}) and sampling times (7, 30 and 90 days)

Our manipulative experiment also showed that PO_4^{3-} content responded to the *C. fluminea* addition as the highest values were observed in *C. fluminea* density of 2000 ind. m^{-2} at day 90 (Fig. 1f). Our results showed that *C. fluminea* carrion might have an effect on PO_4^{3-} by increasing their availability in the soil when present in high densities and similarly to that described for mammalian carrion (Bump et al. 2009b; Parmenter and MacMahon 2009). According to Parmenter and MacMahon (2009), who measured nutrient cycling and decomposition rates in a semiarid shrub-

steppe ecosystem, phosphorous (P) increased in the soil after 15 months of carrion addition, representing up to 18.3% of the total P available in the carcasses. Also, Melis et al. (2007) reported that content of PO_4^{3-} was higher in soils with bison carrion than control soils.

In general, our results were clear enough to recognize that the decomposition of *C. fluminea* carrion can have a significant effect on several soil nutrients increasing their availability and this effect was more effective in some nutrients (e.g. NH_4^+,

NO_2^-, NO_3^- and PO_4^{3-}) than others (e.g. Ca, K and N). However, our results should be interpreted with caution since several factors may affect carrion decomposition rates and may turn the comparisons with other studies challenging. First, the carrion origin and nature: our target species is an invertebrate aquatic species, so it is nutritionally different from most of the studied species available in the literature that usually comprise terrestrial mammals (e.g. pig, sheep, dog, bison, human) (Hopkins et al. 2000; Carter and Tibbett 2006; Carter et al. 2007; Melis et al. 2007; Parmenter and MacMahon 2009). Second, carcass size, which is important in the amount of nutrients able to be transferred into the soil: our study used many small specimens, as opposed to the majority of studies that usually use a unique large specimen (Barton et al. 2013). Third, environmental variables such as moisture, temperature and soil type have important effects on decomposition rates (Forbes et al. 2005; Carter et al. 2007): our manipulative experiment was conducted during summer conditions (high temperature and low precipitation) and in sandy soils. Fourth, belowground activity by vertebrate and invertebrate species: sites may be colonized by different organisms that could exert different controls in the amount of carrion that enters into the soil (Putman 1983; Carter et al. 2007). Besides nutrients, oxygen availability is a major factor controlling microbial decomposition (Medeiros et al. 2009). In our study, we did not measure oxygen availability due to technical difficulties in monitoring this abiotic factor in soil samples; however, future studies should take oxygen availability into account when investigating the drivers of microbial decomposition in soils.

Effects on terrestrial microbial communities

Our study detected differences in fungal biomass only between *C. fluminea* density, with higher values always observed in treatments with a *C. fluminea* density of 2000 ind. m^{-2} (Fig. 2). These results were similar to those obtained by Yang (2004) that showed that the abundance of fungal phospholipid fatty acids (PLFAs) in treatment plots increased 28% after 28 days compared with control plots. Also Bump et al. (2009c) showed that fungal PLFAs were 81% more abundant in the presence of moose carcasses after 40 months compared to control sites. In addition, other studies also showed increased soil fungal

biomass in the presence of carrion (see for example Parkinson et al. 2009).

Furthermore, the soil fungal community showed some succession in response to the decomposition process (Carter and Tibbett 2003; Parkinson et al. 2009). Interestingly, our results of molecular diversity of fungi showed variations in *C. fluminea* density along time mainly noted between day 7 and 90 (Fig. 3a). These results are consistent with the ones obtained by Leff et al. (2015), when investigating the responses of soil microbial communities to nutrient inputs in grasslands across the globe: authors found that N and/or P additions can significantly affect fungal community composition. Although the DGGE technique did not allow us to identify the species involved in the process, the initial decomposition stages usually comprise zygomycetes, deuteromycetes, saprotrophic basidiomycetes and ascomycetes, while ectomycorrhizal basidiomycetes are often present in later stages (Sagara 1992; Yamanaka 1995a, b; Tibbett and Carter 2003). However, this succession was described for time intervals much higher than those used in our experiment, from 1–10 months to 1–4 years (Sagara 1992; Yamanaka 1995a, b). Despite advances made to understand the succession patterns of fungal communities during decomposition (Duarte et al. 2010), the effects of carrion are still largely unknown (Stokes et al. 2009). In an attempt to close this gap, our results demonstrated significant effects on fungal biomass and diversity supporting the idea that the presence of the Asian clam carrion has a significant effect on soil fungal community.

Our experiment also showed that molecular diversity of bacteria varied according to the *C. fluminea* density and over time. Similarly to that found for fungal community, N and/or P additions can also significantly affect bacterial community composition (Leff et al. 2015). Differences in community composition were pronounced for *C. fluminea* densities ≥500 ind. m^{-2} at day 7 (Fig. 3b). This may be partially explained by the high turnover rates of bacteria, which responded rapidly to nutrient addition. Moreover, bacterial composition in plots with *C. fluminea* addition tended to become similar to the control plots at day 90 (Fig. 3b), suggesting that bacterial community recovered over time, after the declining effect of *C. fluminea* carrion. Interestingly, bacterial composition became more similar with control along time for plots with less carrion addition. This suggests that the higher the density of *C.*

fluminea carrion the higher the impact on bacterial community, which would probably take longer to recover. Ecological succession of bacteria during the decomposition of organic matter depends on nutrient availability and undergoes functional and structural changes throughout the decomposition process until complete mineralization (Parkinson et al. 2009; Crippen et al. 2015). However, very few studies have addressed the importance of carrion decomposition on soil microbial communities, mainly on bacteria, and so, additional work is fundamental to better understand the responses of soil bacteria to carrion inputs.

Conclusion

Overall, our results revealed that the decomposition of *C. fluminea* carrion has significant effects belowground, including on nutrients content, fungal biomass and fungal and bacterial diversity. These results are particularly important when viewed across entire landscapes. Indeed, in highly invaded aquatic ecosystems, massive mortalities of *C. fluminea* may change soil chemistry, nutrient cycling and microbial communities even in adjacent terrestrial areas. Although our approach tried to mimic an extreme climatic event resulting from a flood, recent studies showed that massive mortalities of *C. fluminea* also occurred during drought events (Bódis et al. 2014; McDowell et al. 2017) and this situation can also significantly affect aquatic ecosystem functioning. Given the predicted increase and intensification of extreme climatic events (e.g. heatwaves, floods and droughts) and the widespread distribution of several aquatic IAS in the future, the ecological importance of these massive mortalities (and resulting carrion) cannot be ignored and should be investigated in more detail.

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