Food Choice and Fitness of *Folsomia candida* (Collembola, Isotomidae) Fed on Twelve Species of Truffle

Cristina Menta [1]*, Carmine Siniscalco [2], Beatrice Bonati [1] and Sara Remelli [1]

[1] Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, Parma, Italy, [2] Environmental Protection, Monitoring and Biodiversity Conservation Department, National Institute for Environmental Protection and Research-National System for Environmental Protection (ISPRA/SNPA), Rome, Italy

Fungi are a significant food resource for soil fauna, whose grazing behavior can have a significant impact on their development. This relationship is an important aspect in soil functioning, with soil fungi acting as primary agents in decomposition processes. Being one of the most abundant groups among soil fauna, springtails can play a leading role in this context. Despite several previous studies on their epigeous fungal grazing behavior, data regarding the relationship between springtails and truffles are scarce. This study aimed to investigate food preferences of the springtail *Folsomia candida* for grazing on 12 different species of truffles, 11 belonging to *Tuber* genus, and 1 to *Balsamia* genus. We also evaluated how strongly this diet influences survival and reproduction of *F. candida*. In the first experiment, *F. candida* were allowed to choose freely between a cereal mixture (choice test) and 12 different species of truffle. In the second experiment, they were fed on the truffles only (no-choice test) for 28 days. Twelve truffle species were analyzed for survival and reproduction of *F. candida*. *F. candida*'s feeding preference evolved over 72 h, beginning with a strong preference for the control and finally a general preference for truffles. Moreover, Collembola that fed on some *Tuber* species had a lower survival rate and fewer juveniles per adult compared to the control. Compared to other species, *Tuber aestivum* and *Tuber melanosporum*, which are well-known for their ability to produce brûlés, had a positive impact on collembolan fitness, whereas their palatability was not particularly prominent. Hence there was a relationship between diet and fitness in *F. candida*, whilst hardly any relationship was observed between fitness and feeding preference.

Keywords: springtail, *Tuber*, *Balsamia*, food quality, preference test, soil fauna, ectomycorrhizal fungi

INTRODUCTION

The interaction between fungi and soil fauna is a key aspect in soil functioning, since both groups play an important role in the soil food web. Soil fauna has a significant impact on fungi, being the latter an important food resource for it (Hanlon, 1981; Jørgensen et al., 2003; Harold et al., 2005; Rotheray et al., 2011). Interactions between fungi and fungivores influence terrestrial biogeochemical cycles and can also induce fundamental changes in the performance of the plant–fungus association, through which fungal grazers affect mineralization, decomposition rates, and energy transport in soils (Ruess and Lussenhop, 2005; Fernandez et al., 2016).

The grazing of many groups of microarthropods can have either positive or negative effects on fungal communities, depending on the taxon and the abundance of animals (Bengtsson and Rundgren, 1983; Bretherton et al., 2006; Tordoff et al., 2008; Crowther et al., 2012). To prevent detrimental grazing some fungi have evolved defensive strategies, including the presence of crystal structures and other deposits on their hyphal surface, and the production of toxic or distasteful secondary metabolites (Böllmann et al., 2010).

Fungi are an important target in the feeding of springtails, one of the most abundant groups among soil fauna, and grazing by Collembola has produced evident effects on fungal development (Hopkin, 1997; Böllmann et al., 2010), either enhancing or decreasing mycorrhizal spore number (Bakonyi et al., 2002). Thus, their food choice could play a key role in this context. Despite springtails being generalists feeding on a wide range of foods, fungi are one of the main sources for most of them (Parkinson et al., 1979; Jørgensen et al., 2003; Chahartaghi et al., 2005). This group displays marked feeding preference behavior. Moreover, collembolan grazing seems to be species-specific, with selectivity depending on many fungal parameters, such as growing substrate, life stage, mycelium vitality and metabolic activity (Sabatini and Innocenti, 2000; Kaneda and Kaneko, 2004; Heděnec et al., 2013).

Whether the springtails' feeding preference is related to their development and fitness is not clearly known. Some authors (Pfeffer et al., 2010) suggest that *Folsomia candida* (Collembola, Isotomidae) is able to follow an optimal diet for its growth and reproduction rate by choosing to feed on what seems to be its favorite type of fungi. Furthermore, Heděnec et al. (2013) found that the fitness of *F. candida* strongly depended on the litter type rather than on fungal species. Truffles could play a crucial and interesting role here, since their presence influences the biochemical and physical composition of soil, especially in the rhizosphere, where they interact with soil fauna (Callot, 1999; Ricard, 2003; Granetti et al., 2005; García-Montero et al., 2012; Mello et al., 2013; Menta and Pinto, 2016). The genus *Tuber*, representing ectomycorrhizal fungi, produces hypogeous fruiting bodies that release secondary aromatic metabolites as adaptive strategy to attract feeders. This phenomenon is typical of subterranean organisms that strictly depend on animal activity for spore dispersal (Reyna Domenech, 2007). *Tuber* fungi also modify soil biogeochemistry (García-Montero et al., 2009; Trappe and Claridge, 2010) to such an extent that volatile compounds can inhibit the germination and growth of other plants around the host plant (Splivallo, 2008; Menta and Pinto, 2016), generating a burnt area called "brûlé," which could affect soil fauna in many ways. Menta et al. (2014) tried to highlight the differences between soil fauna inside and outside the brûlé resulting from the peculiar environment created by *Tuber aestivum* (Vittadini). The authors showed that some collembolan families were more present in terms of abundance and frequency outside the brûlé, while a species *Folsomia* was abundant inside the brûlé. Some authors (Menta et al., 2014; Pinto et al., 2017) suggested that the conditions created by *T. aestivum* do not have a negative impact on *Folsomia*, which is known to graze on fungi and hyphae (Moore et al., 1985; Thimm and Larink, 1995; Fountain and Hopkin, 2005). Despite previous studies on the epigeous fungal grazing behavior of Collembola, data about the feeding interaction between springtails and truffles are still scarce (Parkinson et al., 1979; Chen et al., 1995; A'Bear et al., 2012; Heděnec et al., 2013).

In this study, we focused on the preference of *F. candida* for grazing on 12 different species of hypogeous truffles, 11 of which belonged to *Tuber*, and 1 to *Balsamia*. We aimed to improve our understanding of the strict relationship between soil fauna and hypogeous fungi by investigating whether the feeding preference influences survival and reproductive performance of *F. candida*. We expect that the fungi that show the greatest palatability influence *F. candida* fitness positively.

MATERIALS AND METHODS

Hypogeous Fungi

We tested 12 species of hypogeous fungi, 11 belonging to *Tuber* and 1 belonging to *Balsamia* genus (**Table 1**). Soil fungi were collected in several municipalities located in Northern and Central Italy, in a period comprised between February 2015 and April 2016. All fungi were provided by ISPRA, the National System for Environmental Protection in Rome and classified and photographed according to their "Mycological biodiversity information system." **Table 1** reports the characteristics of the sites where the fungi were collected, the number of fungi used in the experiments for each species, and the vegetation cover of the areas. The number of truffles for each species varied between three and seven, depending on the availability of truffles during the study period. After collection, the truffle samples were gently brushed, washed with running water to remove soil residues, successively dried at room temperature for 2 h, and classified at species level using a microscope. They were then sliced (3 mm thickness) and dried at 27°C in airflow for 24 h (this treatment may have removed some VOCs but prevented proliferation of molds). The dried samples were placed into separate vacuum bags and sent to Parma University, where they were pulverized using a small grinder and immediately used for the test.

F. candida Cultures

The springtail *F. candida* Willem (Collembola: Isotomidae) is among the most intensively studied of all species of Collembola (Hopkin, 1997). This parthenogenetic species is widely distributed in many environments (Fountain and Hopkin, 2005). Cultures of this species are very easy to maintain and they are excellent for laboratory experiments due to their short reproductive cycle.

The *F. candida* came from 15 laboratory cultures at Parma University. They were reared according to ISO guidelines (ISO 11267, 1999), maintained at 20 (±2°C with 50–55% RH, and fed weekly on a pulverized mixture of dried organic cereals (20% wheat, 20% oats, 20% rye, 20% spelt, and 20% rice). The animals used for egg deposition (aimed to obtain the age-synchronized juveniles used in the test) were collected from all 15 breeding containers and mixed to prevent them originating from a single breeding line.

TABLE 1 | Species of hypogeous fungi used in the study, number of fungi per species, municipality, altitude of the area, and plant species present in the area.

Fungi species	n	Municipality	Altitude (m a.s.l.)	Plant species
Balsamia vulgaris (Vittadini, 1831)	3	Roma	60	*Cedrus atlantica*
Tuber aestivum (Vittadini) Var. *uncinatum* (Chatin) (Montecchi et Borelli, 1995)	7	Piacenza Rieti Roma Viterbo	55–860	*Acer campestre, Acer opalus, Carpinus betulus, Cornus sanguinea, Corylus avellana, Ostrya carpinifolia, Populus alba, Quercus cerris, Quercus ilex, Quercus pubescens, Ulmus minor, Prunus* spp., *Betula* spp., *Prunus laurocerasus*
Tuber borchii (Vittadini, 1831)	7	Roma Viterbo	3–670	*C. atlantica, Fagus sylvatica, Pinus halepensis, Pinus pinea, Q. pubescens*
Tuber brumale (Vittadini, 1831)	5	Rieti Roma Viterbo	55–960	*A. opalus, Juniperus communis, Prunus spinosa, Q. cerris, Q. ilex, Q. pubescens*
Tuber excavatum (Vittadini, 1831)	5	Frosinone Rieti Roma Viterbo	220–750	*A. campestre, C. sanguinea, C. avellana, Fraxinus ornus, O. carpinifolia, Populus alba, Pinus nigra, Q. cerris, Q. pubescens, U. minor*
Tuber fulgens (Quèlet, 1880)	3	Frosinone	750	*P. nigra*
Tuber macrosporum (Vittadini, 1831)	3	Rieti	170–960	*A. campestre, A. opalus, C. avellana, F. ornus, J. communis, O. carpinifolia, P. alba, P. spinosa, Q. cerris, Q. pubescens*
Tuber magnatum (Picco, 1788)	3	Rieti Roma	170–420	*A. campestre, C. sanguinea, C. avellana, F. ornus, O. carpinifolia, P. alba, Q. cerris, Q. pubescens, U. minor*
Tuber melanosporum (Vittadini, 1831)	3	Roma	55	*Q. pubescens*
Tuber mesentericum (Vittadini, 1831)	3	Viterbo	560	*F. sylvatica*
Tuber puberulum (Berkeley and Broome, 1846)	4	Roma Viterbo	60–380	*C. atlantica, Q. cerris, Q. ilex, Q. pubescens*
Tuber rufum (Picco) Var. *rufum* (Montecchi et Lazzari, 1993)	3	Frosinone Roma	60–750	*Q. ilex, P. nigra*

Fungi collected from February 2015 to April 2016. N, number of truffles.

All animals used in the tests were age-synchronized to 10 days by removing eggs from the deposition cultures and, once hatched, inserting juveniles into Petri dishes, with moistened breeding substrate 8:1 (*w/w*) plaster of Paris: activated carbon powder.

Feeding Preference Test

In this experiment *F. candida*'s feeding preference was tested for each fungi species separately with a binary option method consisting in allowing them to choose between a fungus species and the cereal mixture. Cereal mixture was the food used for cultures and during the synchronization phase.

Petri dishes filled with a 0.5 cm plaster layer of Paris mixed with charcoal (8:1) were used for the experiments. Two small hollows (5 × 5 × 3 mm and distant 5 cm one to each other) were made at opposite sides of the Petri dish; one hollow contained 1 gr of a pulverized fungi sample and the other one contained the same quantity of pulverized cereal mixture. There were five replicates for each truffle-control. Ten same age individuals of *F. candida* were transferred from the breeding substrate to the test substrate via an exhauster. No mortality was observed during the process. All the experiments were conducted at 20°C in dark/light 12:12 h conditions. Using a stereomicroscope, the number of *F. candida* feeding on either the truffle or the cereal mixture was checked after 24, 48, and 72 h. The count was made without removing the lid from Petri dishes to avoid Collembola displacement as result of the disturbance.

Survival and Reproduction Test

In this second experiment, survival and reproduction of *F. candida* were tested following the ISO guidelines 11267 (1999). Petri dishes were filled with a 0.5 cm plaster layer of Paris mixed with charcoal (8:1), and one hollow (5 × 5 × 3 mm) in the center was filled with one pulverized fungi sample. Five replicates were prepared for each fungi sample and for the cereal mixture. Ten *F. candida* individuals aged 10–12 days were added to each Petri dish using an exhauster. No mortality events occurred during the process. All experiments were as in Feeding Preference Test, and all fungi were tested at the same time (July, 2016). The Petri dishes were incubated for 28 days and aerated once a week. At the end of this period, the number of surviving adults and the juveniles were recorded using a stereomicroscope with floatation technique (ISO 11267/99).

Statistical Analysis

The Friedman test, followed by the Wilcoxon test for *post-hoc* comparisons, is the non-parametric alternative to the one-way ANOVA with repeated measures which was applied here to evaluate differences in collembolan feeding preference behavior among checks at 24, 48, and 72 h. Interspecific fungi differences on the number of *F. candida* were analyzed using the Kruskal-Wallis test, a non-parametric approach used to compare multiple independent samples, and the Mann-Whitney test with the Bonferroni correction for *post-hoc* analysis. The Wilcoxon test

was used to highlight differences between the *F. candida* grazing on cereal mixture vs. truffle to analyze feeding preference. To evaluate the differences on survival and reproduction between truffles and with cereal mixture, the Dunn test and Bonferroni correction were applied. The Kendall's tau was calculated to investigate the correlation between diet and fitness, in order to test the relationship between n. of *F. candida* grazing on truffles and: (i) n. of juveniles, (ii) n. of juveniles per adults introduced at the beginning, (iii) n. of juveniles per adults survived at the end of the experiment. A *p*-value ≤ 0.05 was considered significant. All statistical analyses were performed using R 3.6.0 software (R Core Team, 2017).

RESULTS

Feeding Preference

The number of *F. candida* feeding on truffles differed between checking hours ($p < 0.001$), with an increasing at 48 h ($p < 0.001$) and 72 h ($p < 0.001$) compared to 24 h (**Figure 1**). *F. candida* showed different responses with different truffle species ($p < 0.001$), and when compared to the cereal mixture. In general, *F. candida* showed a preference for most truffle species except for *T. excavatum* and *T. fulgens* compared to the cereal mixture (**Figure 1**). *T. macrosporum*, *T. magnatum*, and *T. mesentericum* were preferred significantly to the cereal mixture at the end of the experiment (**Figure 1**). In particular, the preference percentage of grazing *F. candida* obtained by *T. macrosporum* was higher than 85%.

Comparing the 12 truffles at 24 h, *T. magnatum* showed a higher number of *F. candida* feeders than *T. aestivum* ($p < 0.01$), *T borchii* ($p < 0.01$), *T. brumale* ($p < 0.001$), *T. puberulum* ($p < 0.05$), and *T. excavatum* ($p < 0.001$); this last truffle also differed from *T. melanosporum* ($p < 0.05$). At 48 h only *T. aestivum* differed significantly from *T. magnatum* ($p < 0.05$), and *T. excavatum* from *T. borchii* ($p < 0.05$). At the end of the experiment (72 h), only *T. macrosporum* showed higher *F. candida* feeders than *T. aestivum*, *T. borchii*, *T. excavatum* ($p < 0.01$ for all), and *T. rufum* ($p < 0.05$).

Survival and Reproduction

Results showed differences between truffles as regards collembolan survival ($p < 0.001$, **Figure 2**). *B. vulgaris, T. aestivum, T. borchii, T. melanosporum, T. mesenterivum*, and *T. puberulum* highlighted a survival percentage close to the cereal mixture (**Figure 2**). *T. excavatum, T. macrosporum* and *T. magnatum* did not differ from control. Differently, *T. rufum* determined significant lower survival when compared with the cereal mixture, but still higher than 50%. On the other hand, *T. brumale* and *T. fulgens* determined survival percentages lower than 50%, and significantly lower than the cereal mixture.

When comparing the truffles, *T. aestivum, T. borchii, T. melanosporum, T. mesentericum*, and *T. puberulum,* led to higher survival when compared with *T. brumale, T. fulgens, T. magnatum*, and *T. rufum*. *T. aestivum* and *T. puberulum* showed higher survival when compared to *T. macrosporum* (**Figure 2**; see **Supplementary Table 1** for statistical significances).

Results on reproduction of *F. candida* highlighted differences in the number of juveniles per adult depending on the species of truffle ($p < 0.001$, **Figure 3**). All truffle species, except *T. borchii, T. melanosporum*, and *T. mesentericum,* led to a lower number of juveniles when compared to the cereal mixture, but the differences resulted significant for *T. fulgens, T. brumale*, and *T. magnatum* only ($p \leq 0.001$ for all).

Comparing truffles, the highest reproduction rate was supported by *T. melanosporum*, significantly different from *T. aestivum, T. brumale, T. fulgens, T. macrosporum, T. magnatum, T. puberulum*, and *T. rufum* (**Figure 2**; see **Supplementary Table 1** for statistical significances).

Instead, *T. fulgens* determined the lowest number of juveniles, differing from all the other species of truffles except for *T. brumale* and *T. magnatum*. Furthermore, although *T. brumale* proved better compared to *T. magnatum*, it caused a significantly lower reproduction rate than the other species of truffle, except for *T. excavatum, T. fulgens, T. macrosporum*, and *T. rufum* (**Figure 2**; see **Supplementary Table 1** for statistical significances).

No correlation was highlighted between diet, in term of n. of *F. candida* grazing on truffles, and fitness (n. of juveniles: tau: −0.039, *p*: not significant; n. of juvenile per adults introduced at the beginning, tau: −0.039, *p*: not significant; n. of juveniles per adults survived at the end of the experiment tau: −0.045, *p*: not significant).

DISCUSSION

The aim of this study was to observe the grazing behavior of *F. candida* fed on twelve species of hypogeous fungi, and to evaluate if different fungi could affect survival and reproduction of this species. Consequently the aim was then to understand how much feeding preference was related to fitness of this collembolan species. It is a well-known fact that *F. candida* can exhibit distinct feeding preferences depending on the fungal species (Tordoff et al., 2008), and this study confirmed both this aspect and the important role of this species in spore dispersion and regulation of fungal community. The interesting addition is that not only do different fungal species have various palatability for this collembolan, but also that fungi were preferred as food resource when the springtails could choose between fungi and the usual food (cereal mixture in this study) that these animals had been used to feeding on for numerous generations. The feeding preference trend observed in this study showed an increment of truffle palatability already 48 h after the beginning of the experiment. This suggests that springtails need a short time to modify their feeding habits. Truffles exhibit their maximum sensorial properties when fresh. With a shelf-life of 7–10 days, truffles quickly lose their flavor intensity and start to spoil (Campo et al., 2017). Recent studies by Splivallo et al. (2015) and Splivallo and Ebeler (2015) show that bacteria associated with truffle-ascocarps and sulfur-containing volatiles, such as thiophene derivates, contribute to truffle aroma. Nevertheless, classical preservation method, like hot air drying (HAD) or dehydration of truffles, reducing
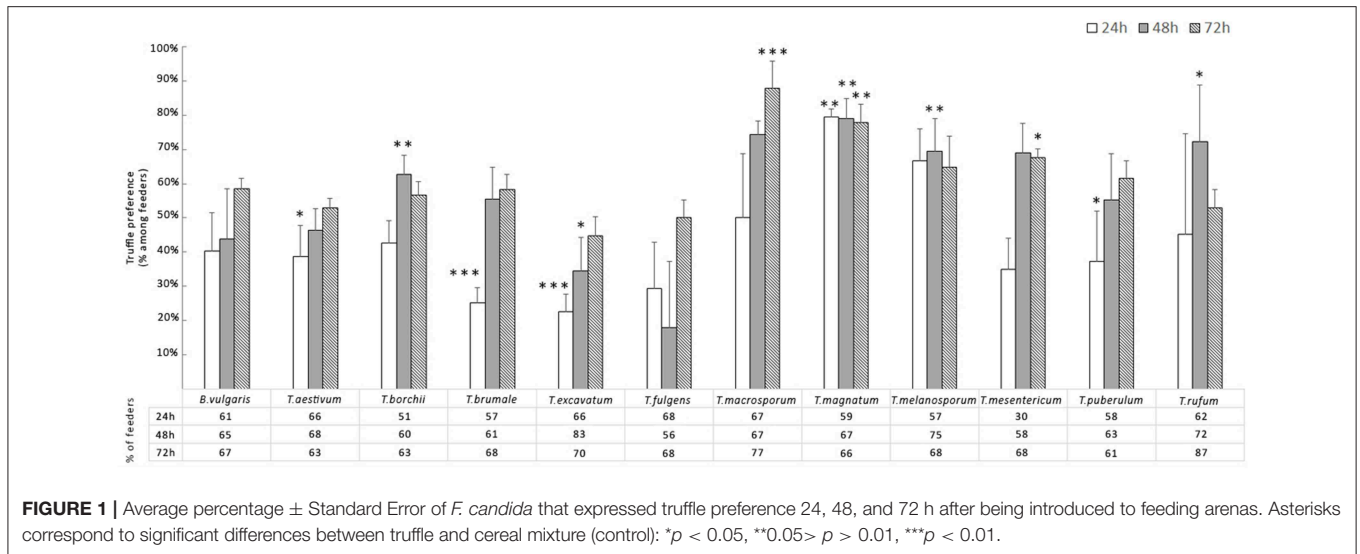
FIGURE 1 | Average percentage ± Standard Error of *F. candida* that expressed truffle preference 24, 48, and 72 h after being introduced to feeding arenas. Asterisks correspond to significant differences between truffle and cereal mixture (control): *$p < 0.05$, **$0.05 > p > 0.01$, ***$p < 0.01$.
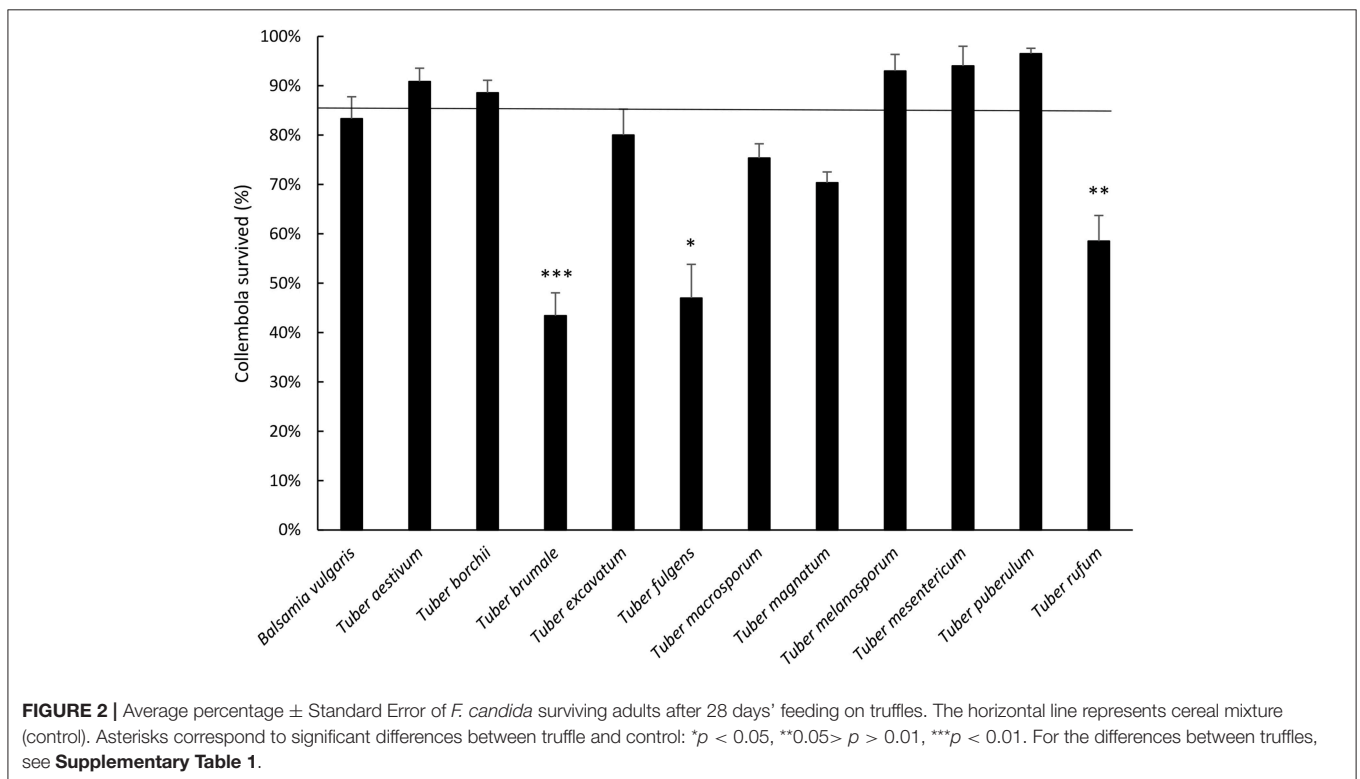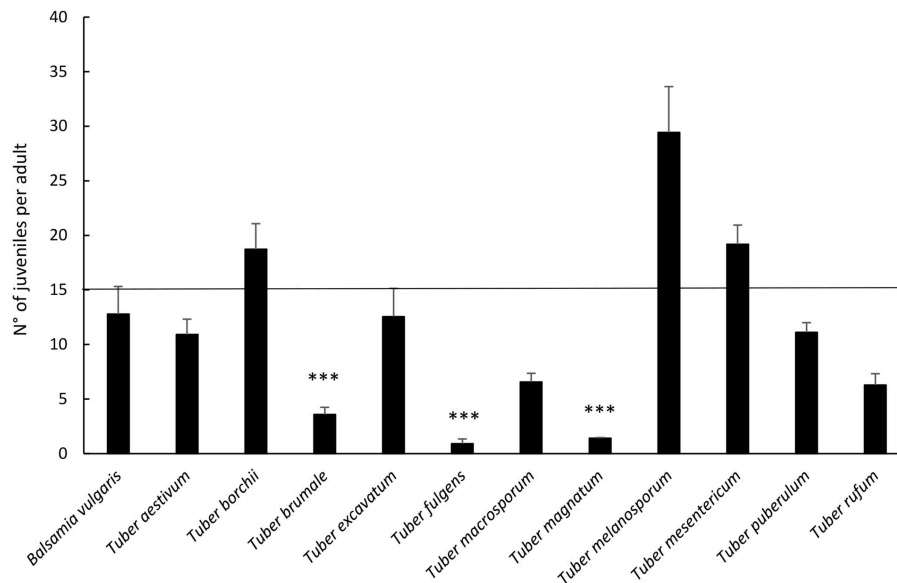

FIGURE 2 | Average percentage ± Standard Error of *F. candida* surviving adults after 28 days' feeding on truffles. The horizontal line represents cereal mixture (control). Asterisks correspond to significant differences between truffle and control: *$p < 0.05$, **$0.05 > p > 0.01$, ***$p < 0.01$. For the differences between truffles, see **Supplementary Table 1**.

water content and microbial growth, slow down enzymatic and chemical activities. The resulting microbial inhibition could partially explain the variations in truffle species preferences often observed throughout the first experiment. Moreover, the rehydration of the dried and pulverized truffle samples could have reactivated the microbiome, which could have served as food resource, being *F. candida* a fungivorous species.

We must however consider that *F. candida* showed variability between fungi. *T. macrosporum* and *T. magnatum* were the two more palatable species, while *T. excavatum* was the least favorite. Truffles attract arthropods with volatile compounds to

facilitate spore dispersion through the grazers' digestive tract and, in this way, compensate their hypogeum condition (Reyna Domenech, 2007). This strategy could be particularly effective on those blind or reduced-eyesight soil-dwelling species that use odors as clues like *F. candida*. The VOCs profiles of *Tuber* spp. are highly complex and are far from being fully described (Vita et al., 2015). Moreover, the geographical origin contributes to the specific variation in VOC profiles, as reported by Üstün et al. (2018) for the white truffle *T. magnatum*. Besides, bacteria associated with truffle-fruiting bodies contribute to truffle aroma, making the system even more complex. In this study, the

FIGURE 3 | Average percentage ± Standard Error of *F. candida* juveniles (numbers of juveniles/surviving adults) after 28 days' feeding on truffles. The horizontal line represents cereal mixture (control). Asterisks correspond to significant differences between truffle and control: *$p < 0.05$, **$0.05 > p > 0.01$, ***$p < 0.01$. For the differences between truffles, see **Supplementary Table 1**.

food source was not characterized in terms of biochemical composition because the focus is on the capacity shown by *F. candida* to discriminate among different truffle species and on the effects of these species on survival and reproduction of *F. candida*. Our results show that there is a substantial variation between species of truffle not only in their palatability but also in the effects on survival and reproduction rate of *F. candida*, in agreement with previous studies (Heděnec et al., 2013). Hubert et al. (2004) suggested the existence of four fungal groups in terms of attractiveness and suitability for grazers' development: (i) preferred and suitable for growth; (ii) preferred, but unsuitable; (iii) avoided, but suitable; and (iv) avoided and unsuitable. Our results fall into the first two categories, since we found that compared with the food provided during breeding, truffle was preferred in many cases but not always suitable for collembolan development. Several authors concluded that collembolans are able to select an optimal diet in order to maximize their fitness (Sabatini and Innocenti, 2000; Jørgensen et al., 2008). However, the current study suggests an inconsistent link, if not a discrepancy, between *F. candida* grazing preference and reproduction, in accord with other experiments, such as Heděnec et al. (2013), where discrepancies between food choice and food suitability emerged. Böllmann et al. (2010) suggested that this repellent characteristic has more influence on feeding preference than fungi palatability. Indeed, *T. magnatum* showed high palatability but a low reproduction rate for *F. candida*, hence easily unsuitable in terms of quality. Furthermore, a link between attractiveness and unsuitability could constitute a mechanism of counterbalance to prevent the damages of overgrazing, since by reducing the reproduction the number of collembola that graze on truffle will be smaller. Considering this hypothesis,

truffles, acting on *F. candida* fitness and collembolan population, can indirectly modify soil biochemical and physical composition and, consequently, change soil microbial community in terms of bacteria and other microorganisms that this species uses as food source. Therefore, the effects of some truffle species are direct, modifying soil biogeochemical properties, and indirect, acting on soil living community and, consequently, on soil food web, organic matter decomposition rate and biogeochemical processes that take place in the soil. The two species of truffles *T. aestivum* and *T. melanosporum* are known for their ability to create brûlés, affecting soil biogeochemistry (García-Montero et al., 2009) and soil fauna community (Menta et al., 2014; Pinto et al., 2017). Our results showed high collembolan survival and reproduction rate for both these species, even if their palatability did not differ significantly from other food resources. In particular, feeding on *T. melanosporum* resulted in the highest reproduction rate observed in this study, supporting Scheu and Simmerling hypothesis 2004 that compounds judged tasteful by Collembola may differ from those useful to enhance their fitness. These results suggest that truffle species able to create brûlés could be potentially valuable resources in terms of fitness for *F. candida*. Menta et al. (2014) proposed that *T. aestivum* metabolites could attract *Folsomia* genus unlike other soil microarthropod taxa that were more abundant outside the brûlés.

In conclusion, our data show that truffle species differed not only in their palatability but also in their effects on fitness of *F. candida*, highlighting an inconsistency between preference and suitability. Other studies should be conducted to understand if the difference in response of *F. candida* was caused by the identity of the metabolites produced by truffle species, and if similar effects are induced on other

fungal grazers. This study aims to stimulate studies for better understand the extremely complex relationship between truffles, microbiome, and soil fauna, not only for extending the scientific knowledges but also for increasing the success in consistent truffle yield. Considering latter aspect, *F. candida* can play an important role in the truffle cultivation, at least for the truffles species that showed both high palatability and fitness for this Collembola.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the ISO 11267:1999.

AUTHOR CONTRIBUTIONS

CM formulated the idea, directed the study, conducted the experiments, and wrote the manuscript. BB helped in the statistical analysis and in the writing of the manuscript. SR conducted the experiments and the statistical analysis, and contributed to writing the manuscript. CS provided and classified all the truffles used in the experiments and participated in the writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2019.00114/full#supplementary-material

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