Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/forestsystems
http://dx.doi.org/10.5424/fs/2014232-04925

Forest Systems 2014 23(2): 349-356
ISSN: 2171-5068
eISSN: 2171-9845

Is truffle *brûlé* a case of perturbational niche construction?

Gilberto Bragato

CRA. Centro per lo studio delle Relazioni Pianta Suolo. Via Triestre, 23. Gorizia, Italy

Abstract

Aim of study: In the context of niche construction theory, the investigation was aimed at assessing if truffle *brûlé* is a case of niche construction by testing if the disappearance of grasses in the *brûlé* induces a non-arbitrary perturbation in the soil physical environment and, in that case, which are the physical processes involved.

Area of study: A natural truffle bed located in the Italian Apennines inside an experimental truffle-producing area of the University of Perugia.

Material and methods: Three aggregate size classes in the soil of a *brûlé* and of the neighbouring grass-covered area were determined with the wet-sieving technique in accordance with international standards. In the first part of the investigation, the area was sampled according to a systematic sampling design and the spatial patterns of aggregate size classes in the *brûlé* and in the neighbouring grass-covered area were compared by means of geostatistics. In the second part, the suppression of grasses in the *brûlé* was mimicked in the laboratory by removing roots from a sample collected in the grass-covered area and the effect of freeze-thaw cycles was tested by comparing a control treatment to other four treatments consisting in sub-samples equilibrated at a water tension of –2.5, –5, –10 and –20 kPa. Samples were submitted to a number of freeze-thaw cycles equal to those recorded in the area, analysed for aggregate size distribution and compared with univariate ANOVA.

Main results: The aggregate size classes larger than 0.25mm displayed a spatial pattern comparable to that of the *brûlé*, with sharp changes along the boundaries of the *brûlé* itself. The laboratory experiment showed that such changes are attributable to freeze-thaw cycles that in one winter season may produce a significant decrease in aggregate size compared to the grass-covered area. Both results indicate that the disappearance of grasses in the *brûlé* fulfils the requirement of perturbational niche construction.

Research highlights: The observed change of soil aggregation in the *brûlé* can be useful in delineating soils suitable for truffle farming and in creating suitable soil physical conditions for truffle production by exploiting freeze-thaw cycles after irrigation in winter.

Key words: aggregates; *brûlé;* niche construction; Tuber; soil structure.

Introduction

Some truffle species – namely *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad., *Tuber indicum* Cooke et Massee and, to a lesser extent, *Tuber mesentericum* Vittad. – have the specific capability to create a circular area with little herbaceous ground cover around the symbiont – an area which is commonly called *brûlé*. Researches on the truffle *brûlé*, mainly focused on *Tuber melanosporum*, have not fully explained yet how it is generated. The main processes involved, perhaps acting together, could be parasitism (Plattner and Hall, 1995), the release of phytotoxic substances (Fasolo-Bonfante *et al.*, 1971; Pacioni, 1991; Splivallo *et al.*, 2011) and/or that of metabolites with allelopathic effects (as suggested by Streiblová *et al.,* 2012).

By considering *brulè* as the ecological niche of the above mentioned truffle species and not merely a way truffles have to mark out their presence in the terrain, I started from the qualitative observation that soil aggregation seems to change if compared to the surrounding grass-covered area. Thanks to the advances in theoretical ecology, I focused my attention on the niche construction theory proposed by Odling-Smee *et al.,* in 2003. Niche construction is the process whereby organisms, through their metabolism and activities, modify both biotic and abiotic components of their own and/or each other's niches. It is perturbational when organisms physically change one or more components of their external environments (Odling-Smee *et al.*, 2003; Kylafis and Loreau, 2011; Odling-

---

* Corresponding author: gilberto.bragato@entecra.it

Received: 30-08-13. Accepted: 04-02-14.

Smee *et al.*, 2013). The following requirements must be fulfilled in niche construction: i) organisms must be actively involved in it; ii) their energy benefits must exceed energy costs of niche construction in the short term; iii) niche construction must enhance fitness in the short term; iv) niche construction must be directed by semantic information whose structure and content is the result of prior natural selection; v) organisms respond to different environmental alternatives on the basis of non-arbitrary criteria, changing for instance the physical state of some selected factors in their environment (Odling-Smee *et al.*, 2003). Truffle *brûlé* satisfies the first four requirements in advance: it is correlated with the presence of *Tuber* species (Napoli *et al.*, 2010); after its generation, truffle species enhance their environmental fitness and have enough energy to produce carpophores; the suppression of grasses, whichever the process involved, is regulated and controlled by genetic information. The question of niche construction can be then reduced to the fulfillment of the last requirement, *i.e.* if the suppression of grasses induce a change in the physical state of environmental factors inside the *brûlé*.

Soil structure is the three-dimensional organization of solids and voids in soil and its modification causes changes in soil physical properties — the flowing of water and air, water holding capacity, erosion, etc.— thus influencing the life cycle of organisms living underground. I specifically considered the change in size of aggregates due to soil freezing and thawing (see Mitchell, 1993; Flerchinger *et al.*, 2005). In brief, as soil freezes, water migration to the freezing front causes ice lenses to form and expand in water-filled pores, thus exerting a pressure on aggregates that can fracture them directly or develop planes of weakness susceptible to future fracturing. The main factors influencing aggregate response to freezing are soil texture, organic matter and soil moisture at the time of freezing (Lehrsch *et al.*, 1991; Kvaerno and Oygarde, 2006). A higher content in clay improves the stability of aggregates smaller than 0.25 mm by increasing the amount of clay-metal bridges. Soil organic matter, on the contrary, mainly stabilizes aggregates of 0.25-2.00 mm by means of humic substances and mucillages, and aggregates larger than 2.00 mm with roots and hyphae (Oades and Waters, 1991). The influence of soil moisture is instead related to the size of intra- and inter-aggregate porosity. When soil moisture is limited, only the former porosity is filled with water, and aggregates are fractured from inside by ice-lenses. If soil moisture rises and water also fills the inter-aggregate space, the pressure of ice lenses outside aggregates counterbalances the internal one, allowing aggregates to resist to fracturing (Mbagwu and Bazzoffi, 1989; Lehrsch, 1998; Dagesse, 2013).

Taking these findings into account, I combined two of the approaches suggested by Boogert *et al.* (2006) —*e.g.* a comparison of naturally occurring sites, with and without the niche constructor, and an experimental simulation of niche construction effects in the absence of the niche constructor— with the purpose: i) to assess if the presence or absence of a *T. melanosporum*/*Q. pubescens brûlé* influences aggregate size classes and their spatial distribution; and, ii) to test if changes in aggregate size were attributable to freeze-thaw cycles and to the soil water content at the time of freezing.

Material and methods

Study area

The investigation was done in an experimental area of the University of Perugia located in the Apennines near Volperino, Central Italy (43° 58' 40" N, 12° 51' 00" E; 750 m a.s.l.). This area is characterized by an average annual temperature of 11.4°C and annual rainfall of 983 mm. The soil is a Rendzic Phaeozem (Skeletic) (IUSS Working Group WRB, 2006) developed on *Scaglia Rossa* formation, a marly limestone of the Cretaceous-Eocene age. Its 40-cm thick A horizon was characterized by a gravel content of about 50% in volume and the fine-earth fraction had the following characteristics: sand 290 g kg^{-1}; silt 240 g kg^{-1}; clay 470 g kg^{-1}; pH 8.3; total organic C 35 g kg^{-1}; $CaCO_3$ 380 g kg^{-1}; cation exchange capacity 52 cmol(+) kg^{-1}.

Soil samples were collected in 1996 in an 18m-size squared area containing a *brûlé* where carpophores were collected in the last years before the investigation, and a grass-covered area. The area was located on a southeast-facing backslope characterized by an 8% slope gradient. Symbiont trees were a couple of old white oaks (*Quercus pubescens* Willd.). Other young white oaks and junipers (*Juniperus communis* L.) were also present in the area (Fig. 1).

Soil sampling and analysis

A number of 36 locations was selected according to a 3 m × 3 m grid. As in this particular area carpopho-

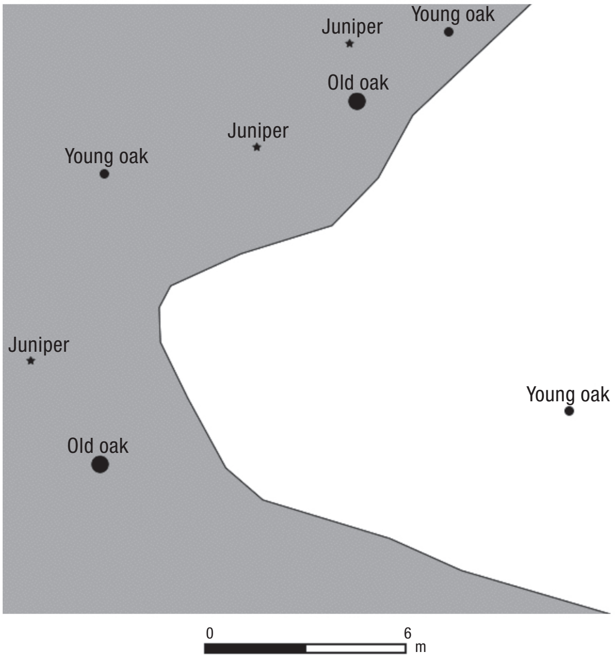

Figure 1. The investigated *brûlé* and, in grey, the grass covered area. Symbionts are the older white oaks present in the area.

res are almost always collected in the first 10 cm of soil, a sample of about 1 kg was collected at a 0-10 cm soil depth at each grid node and then placed in a plastic bag for further laboratory analysis. Samples were air-dried and gently sieved through a 5.66-mm sieve to preserve more than 90% aggregates while discarding coarse gravels. Aggregate size distribution was determined according to the procedure of Kemper and Rosenau (1986). A portion of 40 g was slowly wetted on a sandbox apparatus to a tension of –0.1 kPa. It was then tipped into the top of a nest of five sieves with openings of 4, 2, 1, 0.5 and 0.25 mm and mechanically wet sieved in tap water for 10 minutes at 30 strokes min^{-1}. The fractions retained on sieves were oven-dried and weighed, and aggregate size fractions were calculated as a percentage of the total oven-dry mass.

The suppression of grasses in the *brûlé* was simulated by removing roots from a sample of about 15 kg collected at 0-10 cm in the grass-covered area located just outside the sampling grid. In the laboratory it was sieved at 19.1 mm to remove roots and coarse gravels, and partitioned into 15 sub-samples of 300 g. Three of them were used as a control. Their water content —corresponding to a soil water tension of about –30 kPa— was determined and they were soon after analysed for aggregate size distribution. The remaining ones were put into PVC cylinders of 9.6 cm internal diameter and 5 cm height closed at the bottom with a 150 μm-mesh nylon cloth. Samples were gently pressed on a flat surface, saturated with water on a sandbox apparatus and equilibrated in triplicate at a water tension of –2.5, –5, –10 and –20 kPa that, according to Jurin's law, correspond to water-filled pores with a maximum diameter of 120, 60, 30 and 15 μm respectively. Soil-filled cylinders were randomly placed in a plastic tray and submitted to 17 freeze-thaw cycles at temperatures of –3°C and 20°C. Each cycle lasted 1 to 5 days, for an overall number of frost days corresponding to the annual average recorded in a nearby weather station. At the end of the trial all samples were analysed for aggregate size distribution with the previously described procedure.

Statistical analysis

The first set of data was analysed with a geostatistical approach to compare the spatial pattern of aggregate size parameters with the shape of the *brûlé*. Variogram modelling was done with the restricted maximum likelihood (REML) approach (Lark, 2000; Diggle and Ribeiro, 2007). Since REML requires a Gaussian distribution and none of the variables fulfilled this condition, data were normalized using Gaussian anamorphosis, a procedure based on a transformation function that establishes a correspondence between each one of the sorted raw data and the corresponding frequency quantile in the standardised Gaussian scale (Chilès and Delfiner, 1999). Transformed data were interpolated with Ordinary Kriging or Universal Kriging and back-transformed to raw values through the Gaussian anamorphosis functions previously calculated.

The set of data from the freeze-thaw trial was analysed with univariate analysis of variance. The freeze-thaw effect was considered significant at $p < 0.05$ (F-test) and means were compared with the Tuckey's honestly significant difference (HSD) test. All analyses were performed with *geoR* and *Stats* packages of the R software (R Development Core Team, 2012).

Results

According to Oades and Waters (1991), aggregate sizes were summarized in three classes: 2.00-5.66 mm (large aggregates), 0.25-2.00 mm (medium aggregates) and lower than 0.25 mm (small aggregates). Their

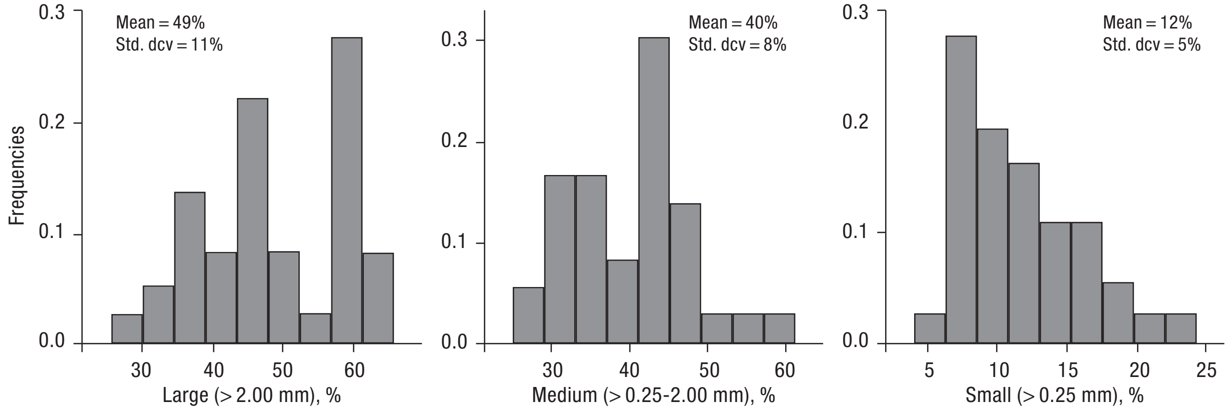

Figure 2. Histograms and summary statistics of aggregate size classes in samples collected for spatial analysis.

distribution and descriptive statistics are reported in Fig. 2. Large and medium size aggregates were inversely correlated with $r = -0.92$. They showed a bimodal distribution with modal maxima at 45% and 60% for large aggregates, and at 35% and 45% for medium ones. Small aggregates were positively skewed and slightly correlated with the other classes. Results of the variogram modelling step are reported in Fig. 3. Variograms of large and medium aggregates displayed Gaussian stationarity (Diggle and Ribeiro, 2007) with a spatial range of 14m and 9m and a random component of variance —the nugget variance— equal to 25% and 9% of the total variance respectively. Small aggregates, on the contrary, showed a non-stationary behaviour, with semivariances increasing in value with the distance (empty circles in Fig. 3c). A linear trend surface was then applied and the variogram model was fitted to semivariances of residuals (full circles in Fig. 3c).

On the basis of variogram modelling, data of large and medium aggregates in unsampled locations were interpolated with ordinary kriging, whereas universal kriging —a kriging interpolation with non-constant mean— was used for small aggregates. The results of the interpolation are shown in the maps of Fig. 4. The spatial pattern of large and medium aggregates is comparable to that of the *brûlé* (Figs. 4a and 4b), the *brûlé* being characterized by a lower content of large aggregates and higher content of medium aggregates than the grass-covered area. Medium aggregates looked more influenced than large ones by the presence of the *brûlé* and indicated an expansion of *brûlé* in the lower left corner of the area not recorded in the survey. The spatial pattern of small aggregates, on the contrary, was not comparable to that of the *brûlé* (Fig. 4c). Values gradually decreased from right to left according to the spatial trend of a non-stationary process already recorded in variogram analysis.

The results of the freeze-thaw trial are shown in Fig. 5, which reports the means and standard errors of the five treatments, along with the results of Tuc-

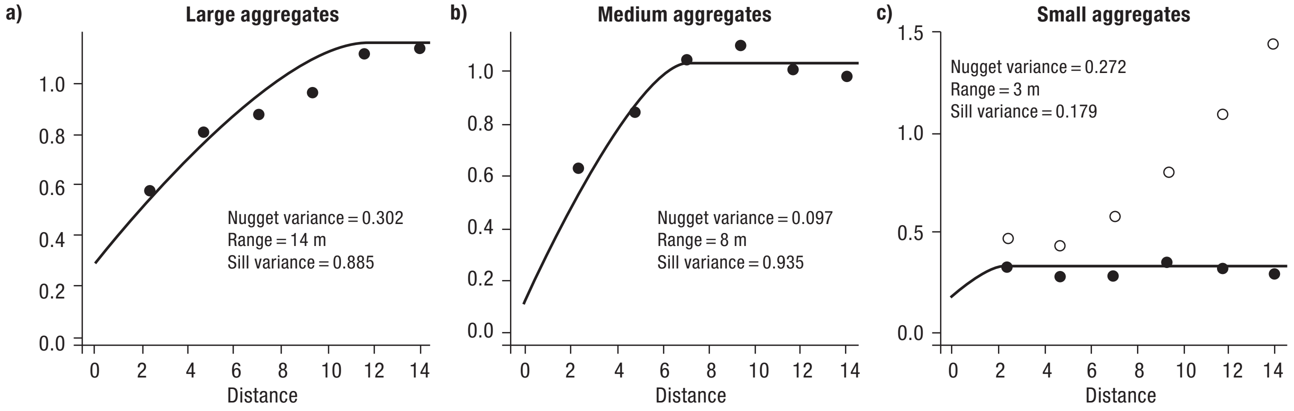

Figure 3. Variograms (circles) and variogram models (bold line) of the three aggregate size classes, along with their quantitative parameters. Nugget variance and sill variance are the random and spatial-dependent components of variance. In Fig. 3c, empty and full circles are variograms of transformed data and of residuals of the trend surface, respectively.

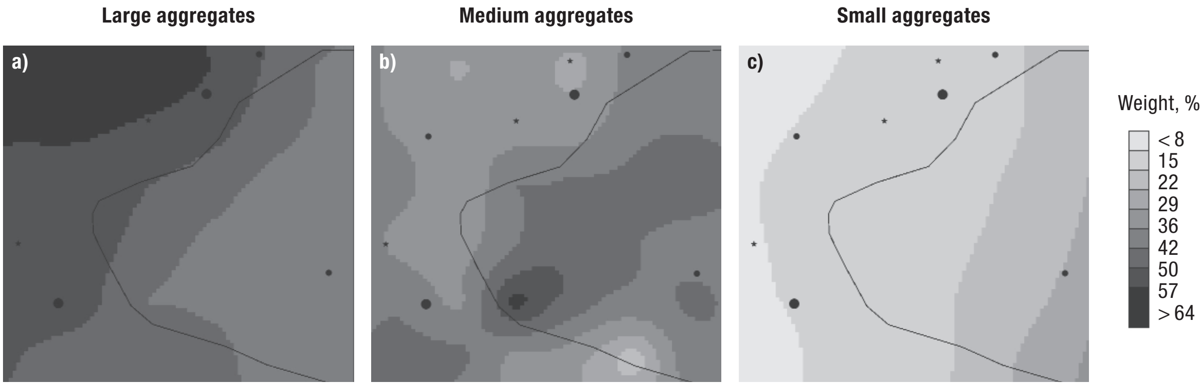

Figure 4. Spatial distribution of the values —expressed as percentages of the total weight— of large (a), medium (b) and small (c) aggregates.

key's HSD test. In large aggregates the –10 kPa and –20 kPa treatments significantly decreased the amount of aggregates compared to the control treatment and the –2.5 kPa treatment, whereas the –5 kPa treatment produced intermediate values between them. This suggests a different effect of freeze-thaw cycles when pores filled with water have a size larger than 120 µm or smaller than 30 µm. Medium aggregates displayed an inverse behaviour, with the control treatment being different from all other treatments. In the same size class the –10 kPa treatment showed the largest difference with respect to the control treatment, but only a marginally significant difference ($p < 0.10$) compared to the –2.5 kPa treatment, thus indicating a lower influence of freeze-thaw cycles on medium aggregates after a single winter season. Small aggregates instead showed the lowest differences between treatments, with scarce effects of freeze-thaw cycles on them.

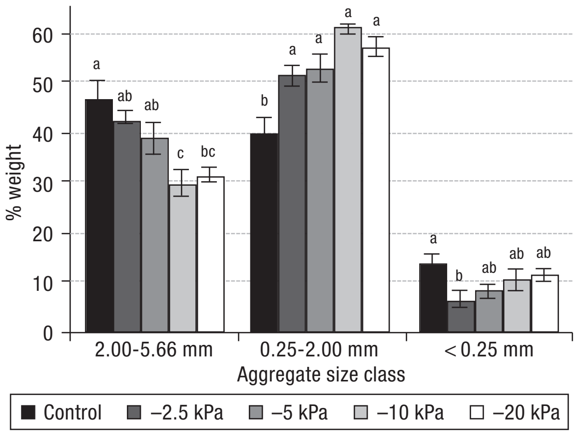

Figure 5. Comparison between the different freeze-thaw treatments in the three aggregate size classes. Data are expressed as percentage of the total weight. Treatments with the same lower case letter are not significantly different at $p < 0.05$ when using the Tuckey's HSD test.

Discussion

Results support the hypothesis of *brûlé* as a case of perturbational niche construction by showing that the suppression of grasses combined with freeze-thaw cycles in winter induce modifications in the degree of soil aggregation and in pore size distribution, thus changing the physical state of soil in the *brûlé*. In the first part of the research the main changes concerned large and medium aggregates. The shape and range of their variograms indicate that they were influenced by environmental factors that are fully effective at the spatial scale of the investigated area. Furthermore, the spatial pattern of these aggregate classes is comparable to the shape of the *brûlé* and they display a relatively sharp change in the area of transition to the grass-covered area, thus suggesting that breakdown processes start working on large aggregates after the suppression of grass cover by the *Tuber* species. Small aggregates, on the contrary, were not affected by the presence of the *brûlé* and variogram analysis suggests they were influenced by processes acting at a much wider spatial scale than that investigated. In aggregates smaller than 0.25 mm solid particles are mainly bound by clay bridges (Oades and Waters, 1991) and, as a consequence, the spatial distribution of small aggregates seems influenced by soil forming factors producing local changes in clay content and/or type.

In the laboratory experiment, freeze-thaw cycles modified the amount of the three aggregate size classes in agreement with the results of the field investigation: the percentage of medium aggregates increased at the expense of large ones, whereas small aggregates were only slightly affected by freezing. The experiment also suggests that a significant decrease in aggregate size can be obtained after the first winter season following the disappearance of grasses. The moisture content at the beginning of the experiment played a significant role in the breakdown of large aggregates. Compared to the control treatment, the largest and most significant decrease in the amount of large aggregates was obtained when 30 µm-pores were filled with water. This result and the non-significant decrease recorded with 120 µm water-filled pores indicate a size threshold of 30-60 µm between inter-aggregate and intra-aggregate pores. Below such threshold ice lenses are effective in the aggregate breakdown process.

Since ice lenses widen intra-aggregate pores, freeze-thaw cycles not only fracture large aggregates, but also increase bulk soil volume, and this explains the slight rise of the soil surface often observed in the *brûlé* at the end of the winter season. Dagesse (2010) found that the increase in soil volume increase takes place at a soil moisture content exceeding 74-90% of soil water saturation. Combining the lower of this pair of values with the data of the freeze-thaw trial, the lowest size of water-filled pores at which soil volume starts to increase could be set at about 12 µm. This size, slightly lower than that of 15 µm corresponding to the –20 kPa treatment, is in line with the results of Fig. 5 and suggests that freeze-thaw cycles increase soil porosity when water-filled intra-aggregate pores range from about 12 µm to less than 60 µm in size, a class of pores playing a leading function in the exchange of air and water between soil and the atmosphere (Pagliai and Vignozzi, 2002). From the niche construction point of view, the modifications of soil structure recorded in the *brûlé* change the physical state of the environment of *Tuber* species. The increase of soil porosity, in particular, enhances the rate of oxygen exchanged with the atmosphere, making the growth of *Tuber* species easier.

This investigation also provides information on the type of soil required by *brûlé*-producing *Tuber* species to construct their niche. According to Flerchinger *et al.* (2005), soil structure both inside and outside the *brûlé* is very stable thanks to the combination of high clay content and a soil organic matter content larger than 30 g kg^{-1}, the latter being the major binding agent of aggregates larger than 0.25mm (Oades and Waters, 1991). The combination of such stability with the effect of freeze-thaw cycles on large and medium aggregates, and the quite constant amount of small aggregates in the range of 10-20% in weight suggest that when the *Tuber* species weaken or stop the control on grasses, the new network of grass roots and the organic substances they excrete help soil structure to turn back to the level of organization present before the creation of the *brûlé*.

If we adopt the criteria suggested by Lal (1997) and we consider *brûlé* as a natural perturbation, the soil suitable for *brûlé* can be considered highly resilient, being characterized by a high buffering capacity and a high rate of recovery to the state before perturbation. The resiliency of soils suitable for *Tuber* species has practical implications for the delineation of areas addressed to truffle farming. Since resilience is inversely related to degradation, soils suitable for the farming of *brûlé*-producing truffles should not be affected by the loss of particles and aggregates. When particles and aggregates smaller than 0.25 mm released by freeze-thaw fracturing are removed by erosion or mass movement, soil gets thinner with the consequence that carpophores grow nearer to the surface and the soil environment becomes dryer because of the decrease in its water holding capacity. In practical terms, the delineation of areas suitable for truffle production could be done in three steps: i) selection of the subset of soils unaffected by erosion/resedimentation with the help of geomorphological approaches; ii) further narrowing of potentially suitable areas by combining working soil chemical properties with physical characteristics —structure grade and rupture resistance, bulk density, etc.— that are related to soil structure and compactness; and, iii) determination of the water-stability of soil aggregates for the final selection of areas actually suitable for truffle production.

Also truffle farming may capitalize on the resiliency of soils suitable for *brûlé*-producing *Tuber* species. The freeze-thaw effect in the *brûlé* is comparable to that of soil tillage, done to aerate the soil of the *brûlé* and to eventually cut symbiont roots for the renewal of mycorrhizal apexes. In truffle orchards equipped with an irrigation system, an irrigation in January aimed at rising soil moisture to a tension of –10 kPa could exploit the frost days of late winter to increase aggregate breakdown, thus limiting the need for more expensive tillage practices in particular inside the *brûlé*.

Conclusions

The disappearance of grasses in the *brûlé* induces modifications of soil structure that are determined by freeze-thaw cycles taking place in the winter season. Aggregates larger than 0.25 mm are fractured by ice lenses growing in intra-aggregate pores of 12-60 µm size. A related result is the increase in volume of this class of pores, which play a leading function in the exchange of water and air between soil and the atmosphere. From the point of view of obligate aerobes like *Tuber* species, these modifications enhance the rate of oxygen that can be exchanged with the atmosphere, making their growth in soil easier.

The same changes fulfil the requirement of the niche construction theory for modifications in the physical state of selected environmental factors to discriminate niche-constructing organisms. The results obtained not only confirm the initial hypothesis of *brûlé* as a case of perturbational niche construction, but also suggest that areas suitable for *brûlé*-producing *Tuber* species are highly resilient in terms of soil structure. We can take advantage of such resilience to delineate areas suitable for truffle farming with more accuracy and to manage truffle orchards with winter irrigation in order to get results comparable to those of soil tillage.

Acknowledgements

I would like to thank Prof. M. Bencivenga for permission to sample the investigated area and the helpful information about it. I also thank Dr. Luciano Lulli and Dr. Tiziano Panini for the deep discussion about soils suitable for truffle growth and the specific features of soil structure in the *brûlé*.

References

Boogert NJ, Paterson DM, Laland KN, 2006. The implications of niche construction and ecosystem engineering for conservation biology. Bioscience 56: 570-578.

Chilés JP, Delfiner P, 1999. Geostatistics: modeling spatial uncertainty. Wiley, New York.

Dagesse DF, 2010. Freezing induced bulk soil volume changes. Can J Soil Sci 90: 389-401.

Dagesse DF, 2013. Freezing cycle effects on water stability of soil aggregates. Can J Soil Sci 93: 1-11.

Diggle PJ, Ribeiro Jr PJ, 2007. Model-based geostatistics. Springer, Berlin, Germany.

Fasolo-Bonfante P, Fontana A, Montacchini F, 1971. Studi sull'ecologia del *Tuber melanosporum*. Demostrazione di un effetto fitotossico. Allionia 17: 47-54.

Flerchinger GN, Lehrsch GA, McCool DK, 2005. Freezing and thawing processes. In: Encyclopedia of soils in the environment (Hillel D, ed). Elsevier, Oxford, UK. pp: 104-110.

IUSS Working Group WRB, 2006. World reference base for soil resources 2006, 2nd edition. World Soil Resources Reports No, 103. FAO, Rome, Italy.

Kemper WD, Rosenau RC, 1986. Aggregate stability and size distribution. Methods of soil analysis. Part 1 (Klute A, ed). ASA, Madison, WI, USA.

Kvaerno SH, Oygarden L, 2006. The influence of freeze-thaw cycles and soil moisture on aggregate stability of three soils in Norway. Catena 67: 175-182.

Kylafis G, Loreau M, 2011. Niche construction in the light of niche theory. Ecol Lett 14: 82-90.

Lal R, 1997. Degradation and resilience of soils. Phil Trans R Soc Lond B 352: 997-1010.

Lark RM, 2000. Estimating variograms of soil properties by the method-of-moments and maximum likelihood. Eur J Soil Sci 51: 717-728.

Lehrsch GA, Sojka RE, Carter DL, Jolley PM, 1991. Freezing effects on aggregate stability affected by texture, mineralogy, and organic matter. Soil Sci Soc. Am J 55: 1401- 1406.

Lehrsch GA, 1998. Freeze-thaw cycles increase near-surface aggregate stability. Soil Sci 163: 63- 70.

Mbagwu JSC, Bazzoffi P, 1989. Effects of antecedent matric potential on the stability of soil aggregates subjected to cyclic freezing and thawing as evaluated by three structural indices. Soil Technol 2: 59-70.

Mitchell JK, 1993. Fundamentals of soil behaviour. Wiley, New York, USA.

Napoli C, Mello A, Borra A, Vizzini A, Sourzat P, Bonfante P, 2010. *Tuber melanosporum*, when dominant, affects fungal dynamics in fruffle grounds. New Phytol 185: 237-247.

Oades JM, Waters AG, 1991. Aggregate hierarchy in soils. Aust J Soil Res 29: 815-828.

Odling-Smee FJ, Laland KN, Feldman MW, 2003. Niche construction. The neglected process in evolution. Princeton University Press, Princeton, NJ, USA.

Odling-Smee J, Erwin DH, Palkovacs EP, Feldman MW, Laland KN, 2013. Niche construction theory: a practical guide for ecologists. Q Rev Biol: 88, 3-28.

Pacioni G, 1991. Effects of *Tuber* metabolites on the rhizospheric environment. Mycol Res 95: 1355-1358.

Pagliai M, Vignozzi N, 2002. Image analysis and microscopic techniques to characterize soil pore system. In: Physical methods in agriculture. Approach to precision and quality (Blahovec J, Kutílek M, eds). Springer Science + Business Media, New York. pp: 13-38.

Plattner I, Hall IR, 1995. Parasitism of non-host plants by the mycorrhizal fungus *Tuber melanosporum*. Mycol Res 99: 1367-1370.

R Development Core Team, 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available in http://www.R-project.org/.

Splivallo R, Ottonello S, Mello A, Karlovsky P, 2011. Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol 189: 688-699.

Streiblová E, Gryndlerová H, Gryndler M, 2012. Truffle *brûlé:* an efficient fungal life strategy. FEMS Microbiol Ecol 80: 1-8.