Artificial-turf surfaces for sport and recreational activities: microbiota analysis and 16S sequencing signature of synthetic vs natural soccer fields

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ABSTRACT

Synthetic fibres are used in place of the natural grass worldwide, for realizing playgrounds, soccer fields and even domestic gardens or recreational structures. An intensive use of artificial turf is currently observed in sports facilities, due to lower costs, higher sustainability in recycling of materials, and advantages related to athletic practice and performance. However, even if chemical and physical risks were studied, the microbiological component was not fully addressed, especially considering a comprehensive evaluation of the microbiota in synthetic vs natural playground surfaces. Here, we investigated the microbial community present on soccer fields, using Next Generation Sequencing and a 16S amplicon sequencing approach. Artificial and natural turfs show own ecosystems with different microbial profiles and a mean Shannon's diversity value of 2.176 and 2.475, respectively. The bacterial community is significantly different between facilities (ANOSIM: R = 0.179; p < 0.001) and surface materials (ANOSIM: R = 0.172; p < 0.005). The relative abundance of potentially pathogenic bacterial OTUs was higher in synthetic than in natural samples (ANOVA, F = 2.2). Soccer fields are characterized by their own microbiota, showing a different 16S amplicon sequencing signature between natural and artificial turfs.

1. Introduction

Artificial turfs are surfaces of synthetic fibres more and more frequently used in place of the natural grass (Watterson, 2017; Fleming, 2011). Synthetic pitches are commercialized worldwide for realizing playgrounds, soccer, football or rugby fields and even in domestic gardens or recreational structures. Their successful diffusion is supported by lower costs, higher sustainability in materials reuse, water saving, and other advantages related to athletic practice and performance (Sánchez-Sánchez et al., 2018; Burillo et al., 2014). Starting from the 60s several synthetic materials were developed, and the technology was continuously and globally evolving. In recent years, several raw resources were used as a backing from jute to nylon or polypropylene, but these surfaces were considered too stiff and abrasive (Sandkuehler et al., 2010). The third generation of turfs was introduced in the 2000s and is characterized by long and less densely packed tufts fibres as well as an infill comprising elastomeric material, such as crumb rubber (Severn et al., 2011; Emery et al., 2016). The synthetic turfs are assembled with natural products such as cork or coir, a coconut-derived material, sand and crumb rubber as “soil” or “infill”, but not grass substitutes (Houling et al., 2014). Therefore, a continuous progress in the field generated very different matrices that were extensively applied as pitches for different uses in place of natural grass.

The intensive diffusion of this technology in sport facilities raised several doubts on possible health risks, both injuries, chemical and microbiological hazards for users and environments (Watterson, 2017; Perkins et al., 2019). Recent studies have showed higher rates of abrasion injuries on artificial turf surfaces compared to natural grass playing fields (Twomey et al., 2018; Meyers, 2013; Williams et al., 2016). The debate about chemical hazards of artificial pitches and playgrounds is very current topic between manufacturers, suppliers, purchasers, workers and users of different playgrounds (Etma, 2016; Moore, 2014). Several studies have identified potential hazards associated with synthetic turf such as heavy metals, volatile organic compounds, polycyclic aromatic hydrocarbons including benzopyrenes and phthalates (Anderson et al., 2006; Perkins et al., 2019; Celeiro et al., 2018). However, hazards...
associated with artificial turfs were not definitely confirmed and scientists, public health operators, consumers or environment agencies still continue to provide additional updates on studies or regulations (Macfarlane et al., 2015; USEPA, 2016; EHHI, 2016). The microbiological risk has been less investigated, even if several studies raised a possible association between turf burns and infections in injured athletes, identifying the synthetic turf as a possible source of pathogens, including community-acquired methicillin-resistant *Staphylococcus aureus* and other antibiotic resistant microorganisms (CDC, 2003; Kirkland and Adams, 2008; Cohen, 2008). It was suggested that the turf infill may represent a favourable niche for the accumulation and selection of bacteria species, especially if maintenance is not regularly and appropriately performed (Bass and Hintze, 2008).

Here, we report a description of the microbiological profile of synthetic vs natural turfs used on soccer fields, suggesting possible applications in safety and management issues.

### 2. Methods

#### 2.1. Study design and sampling

We collected a total of 51 samples from 11 facilities in the area of Rome, involving 7 artificial and 5 natural FIFA-regulatory fields. Of these, 11 samples (collected from 3 of these facilities) were excluded because did not pass the quality controls due to low quantity or low quality in the isolated DNA. Flocked swabs (FS) with the active drying system (model 4N6FLOQSwabs, Copan) were used to obtain samples (n = 40). At each soccer field, three points were sampled: center mark (CM), penalty area (PA), corner arc (CA). Each site was swabbed for between 10 and 15 s. Moreover, grass/sand (GS) samples (n = 11), approximately 3–10 mg, were directly collected in the lateral play area. Due to low quantity or quality in DNA extraction, 11 samples were excluded (10 FS and 1 GS sample). Failed DNA isolation or amplification may have been due to multiple factors: e.g. low DNA concentrations, presence of PCR inhibitors (i.e. humic substances, phenolic compounds, pesticides, detergents, etc.). Finally, we performed the study on 40 samples that were processable: 27 from artificial and 13 from natural soccer fields, respectively corresponding to 7 artificial and 3 natural soccer fields. Table 1 reports descriptive metadata collected for each sample, including date, location and climate in the time of sampling. Samples were transferred on ice to laboratory (within 24 h) and stored at 4 °C until to extraction.

#### 2.2. DNA extraction and purification

After sample collection, each swab was inserted into the semi-permeable NAO Baskets and was broken inside at the breakpoint. The samples were pretreated with glass beads® (Sigma Aldrich, USA) and 200 μl of Lysozyme Solution® (Sigma Aldrich, USA), adapting the protocol as previous described (Valeriani et al., 2017, 2018a and b). In a second phase we followed the standard protocol procedure of GenElute® Bacterial Genomic DNA Kit (Sigma Aldrich, USA). For GS, the DNA extraction by pellets was performed with GenElute® Bacterial Genomic DNA Kit, following the manufacturer’s instruction. Sterile swabs were used as extraction and amplification controls.

#### 2.3. 16S amplicon sequencing

Samples were prepared according to the "16S Metagenomic Sequencing Library Preparation" guide (Part# 15044223 rev. A; Illumina, San Diego, CA, USA). Briefly, the primers containing Illumina adapter and linker sequence and targeting the V1–V2 regions of bacterial 16S rRNA genes were used (Valeriani et al., 2018b; Wen et al., 2017). Three libraries with unique tags were generated for each sample as technical replicates. Each amplification reaction had a total volume of 25 μl containing 12.5 μl of KAPA HiFi HotStart ReadyMix (Roche,

### Table 1

Descriptive metadata was collected for each sample, including date, location and climate in the time of sampling.

| ID  | Sport Facilities | Sampling Time | Material            | Sampling point     |
|-----|------------------|---------------|---------------------|--------------------|
| C1  | I                | nov-16        | Synthetic turf      | Center Mark        |
| C2  | I                | nov-16        | Synthetic turf      | Corner Arc         |
| C13 | I                | nov-16        | Synthetic turf      | Grass/sand play area|
| C3  | II               | nov-16        | Synthetic turf      | Center Mark        |
| C4  | II               | nov-16        | Synthetic turf      | Corner Arc         |
| C5  | II               | nov-16        | Synthetic turf      | Penalty Area       |
| C14 | II               | nov-16        | Synthetic turf      | Grass/sand play area|
| C12 | II               | nov-16        | Natural grass       | Center Mark        |
| C37 | II               | Jan-18        | Natural grass       | Center Mark        |
| C38 | II               | Jan-18        | Natural grass       | Penalty Area       |
| C39 | II               | Jan-18        | Natural grass       | Corner Arc         |
| C49 | II               | Jan-18        | Natural grass       | Grass/sand play area|
| C6  | III              | nov-16        | Synthetic turf      | Center Mark        |
| C7  | III              | nov-16        | Synthetic turf      | Corner Arc         |
| C8  | III              | nov-16        | Synthetic turf      | Penalty Area       |
| C15 | III              | nov-16        | Synthetic turf      | Grass/sand play area|
| C9  | IV               | nov-16        | Synthetic turf      | Center Mark        |
| C10 | IV               | nov-16        | Synthetic turf      | Corner Arc         |
| C11 | IV               | nov-16        | Synthetic turf      | Penalty Area       |
| C16 | IV               | nov-16        | Synthetic turf      | Grass/sand play area|
| C28 | V                | Jan-18        | Synthetic turf      | Center Mark        |
| C29 | V                | Jan-18        | Synthetic turf      | Penalty Area       |
| C30 | V                | Jan-18        | Synthetic turf      | Corner Arc         |
| C46 | V                | Jan-18        | Synthetic turf      | Grass/sand play area|
| C31 | VI               | Jan-18        | Synthetic turf      | Center Mark        |
| C32 | VI               | Jan-18        | Synthetic turf      | Penalty Area       |
| C33 | VI               | Jan-18        | Synthetic turf      | Corner Arc         |
| C47 | VI               | Jan-18        | Synthetic turf      | Grass/sand play area|
| C34 | VII              | Jan-18        | Synthetic turf      | Center Mark        |
| C35 | VII              | Jan-18        | Synthetic turf      | Penalty Area       |
| C36 | VII              | Jan-18        | Synthetic turf      | Corner Arc         |
| C48 | VII              | Jan-18        | Synthetic turf      | Grass/sand play area|
| C40 | VIII             | Jan-18        | Natural grass       | Center Mark        |
| C41 | VIII             | Jan-18        | Natural grass       | Penalty Area       |
| C42 | VIII             | Jan-18        | Natural grass       | Corner Arc         |
| C50 | VIII             | Jan-18        | Natural grass       | Grass/sand play area|
| C43 | IX               | Jan-18        | Natural grass       | Center Mark        |
| C44 | IX               | Jan-18        | Natural grass       | Penalty Area       |
| C45 | IX               | Jan-18        | Natural grass       | Corner Arc         |
| C51 | IX               | Jan-18        | Natural grass       | Grass/sand play area|
A total of 2,268,361 sequences were generated from 40 samples (Table 1 and detailed results of the amplicon sequencing analysis are shown in Table 2). The number of sequences for each sample ranged from

![Fig. 1](image-url) Rarefaction curves calculated for each sample, showing an adequate and reliable sampling and sequencing effort for describing the bacterial community.
6.69% in synthetic and natural turfs, respectively. We performed the
analysis of the significance of alpha diversity measures between sample
groups (Fig. 2). Overall, there was no specific trend detected in the
richness and diversity of taxa between samples. Indeed, as determined by
the Shannon species diversity and the Evenness index, the complexity of
bacterial communities varied slightly between type of materials. The
different sampling points collected from natural fields show very nar-
rower confidence interval, except for the CM point. The mean Shannon's
diversity values for the synthetic soccer fields exhibited slightly higher
diversity level (Natural: $H = 2.176 \pm 0.456$ and $E = 0.33 \pm 0.06$; Syn-
thetic $H = 2.475 \pm 0.161$ and $E = 0.38 \pm 0.088$). Bacterial community
composition was significantly different between facilities (ANOSIM: $R = 0.179$; $p < 0.001$). Similar results were obtained using the Principal
Component Analysis (PCA) to assess clustering (and potential separation)
of facilities (Fig. 3a). The bacterial community pooled by the different
types of materials was significantly different ($R = 0.172$; $p < 0.005$).
The partial least square-discriminant analysis (PLS-DA) depicts a Pearson
distance showing a separation between the natural and synthetic soccer
field, suggesting a noticeable shift in community structures among the
two groups of turfs (Fig. 3b). The analysis of similarities trough ANOSYM
between several sampling points showed no diversification between the
points in the same location within the field (e.g. all CM respect to all CA
or PA; ANOSIM: $R = 0.161$; $p > 0.6$). However, when performing the
analysis within the same facility we can detect a dissimilarity between
each of the different locations (e.g. CM, CA or PA; ANOSIM: $R = 0.513$; $p < 0.001$), suggesting that the microbiota composition may consistently
be influenced by both the facility and the location within the playground.

Regarding the observed genera, *Chryseobacterium* was the predomi-
nant one, accounting for 8.8% of total effective bacterial sequences in all
samples, both synthetic and natural (Figs. 4 and 5). Other dominant
genera include other environmental bacteria such as *Flavobacterium* (5%) and
*Pedobacter* (3.6%). However, when samples were grouped by type of
turf, synthetic kinds harbored a different microbiota (Fig. 5). In these
samples median UniFrac distances were larger, and the distributions
were wider, suggesting that the beta-diversity variance was significantly
greater and probably in

| Samples | Number Reads (Passing filter) | Shannon (Species Diversity) | Number of Species (Identified) | Evenness |
|---------|-------------------------------|-----------------------------|--------------------------------|----------|
| C1      | 250,451                       | 2.784                       | 746                            | 0.42     |
| C2      | 170,372                       | 2.882                       | 684                            | 0.44     |
| C3      | 218,213                       | 2.927                       | 778                            | 0.44     |
| C4      | 260,439                       | 3.078                       | 861                            | 0.46     |
| C5      | 203,130                       | 3.121                       | 795                            | 0.47     |
| C6      | 95,282                        | 3.011                       | 779                            | 0.45     |
| C7      | 143,017                       | 2.855                       | 598                            | 0.45     |
| C8      | 145,551                       | 2.908                       | 824                            | 0.43     |
| C9      | 27,268                        | 2.803                       | 495                            | 0.45     |
| C10     | 69,188                        | 2.583                       | 590                            | 0.40     |
| C11     | 133,129                       | 2.589                       | 688                            | 0.40     |
| C12     | 53,454                        | 3.142                       | 917                            | 0.49     |
| C13     | 108,481                       | 3.182                       | 690                            | 0.49     |
| C14     | 73,499                        | 3.025                       | 740                            | 0.46     |
| C15     | 91,324                        | 3.203                       | 758                            | 0.48     |
| C16     | 218,172                       | 3.268                       | 836                            | 0.49     |
| C17     | 471,205                       | 2.145                       | 1181                           | 0.30     |
| C18     | 195,921                       | 2.190                       | 886                            | 0.32     |
| C19     | 145,530                       | 1.935                       | 789                            | 0.29     |
| C20     | 58,667                        | 1.832                       | 502                            | 0.29     |
| C21     | 29,025                        | 2.128                       | 507                            | 0.34     |
| C22     | 136,279                       | 2.516                       | 843                            | 0.37     |
| C23     | 105,569                       | 2.642                       | 696                            | 0.31     |
| C24     | 80,684                        | 1.967                       | 640                            | 0.30     |
| C25     | 148,085                       | 1.576                       | 758                            | 0.24     |
| C26     | 39,718                        | 1.328                       | 435                            | 0.22     |
| C27     | 36,546                        | 1.943                       | 476                            | 0.32     |
| C28     | 106,348                       | 1.243                       | 579                            | 0.20     |
| C29     | 113,812                       | 2.132                       | 654                            | 0.33     |
| C30     | 85,091                        | 1.810                       | 555                            | 0.29     |
| C31     | 191,711                       | 1.986                       | 802                            | 0.30     |
| C32     | 66,060                        | 1.913                       | 543                            | 0.30     |
| C33     | 126,100                       | 2.871                       | 1125                           | 0.41     |
| C34     | 85,696                        | 2.107                       | 761                            | 0.32     |
| C35     | 105,308                       | 2.115                       | 837                            | 0.31     |
| C36     | 111,098                       | 1.688                       | 633                            | 0.26     |
| C37     | 66,734                        | 1.864                       | 481                            | 0.30     |
| C38     | 83,162                        | 2.209                       | 714                            | 0.24     |
| C39     | 58,294                        | 2.511                       | 727                            | 0.38     |
| C40     | 105,428                       | 1.819                       | 690                            | 0.28     |

27,268 to 471,205 leading to the identification of 1181 OTUs defined at
97% identity. Rarefaction curves were calculated for each sample
(Fig. 1), showing an adequate and reliable sampling and sequencing
effort for describing the bacterial community (Wen et al., 2017).
Interestingly, several reads resulted as unknown representing 21.8% and
6.69% in synthetic and natural turfs, respectively. We performed the

Table 2
Summary of NGS analysis after quality assessment of sequences.

![Fig. 2. A box plot presentation of the distribution of Shannon and Evenness indices of the four groups in natural and synthetic turf.](42x68 to 553x249)
abundance: 2 %, 2 %, 1.3 %, respectively). Conversely, the natural surfaces were found to have a microbial community structure much more comparable to those present in phyllosphere environments, with the lowest median UniFrac distance between samples and a narrow distribution of these distances. *Chryseobacterium* was observed in all samples but more present on artificial mats (16.2 % relative abundance versus <0.1 in natural turf; p value < 0.001). In natural turfs, *Flavobacterium*, *Janthinobacterium*, *Brevundimonas*, *Agrobacterium* and *Bacillus* (relative abundance: 11.1 %, 7.8 %, 6.4 %, 5.9% and 4.7%, respectively) were also detected.

The analysis of potentially pathogenic genera resulted in a higher relative abundance of OTUs corresponding to opportunistic bacteria abundance: 2 %, 2 %, 1.3 %, respectively). Conversely, the natural surfaces were found to have a microbial community structure much more comparable to those present in phyllosphere environments, with the lowest median UniFrac distance between samples and a narrow distribution of these distances. *Chryseobacterium* was observed in all samples but more present on artificial mats (16.2 % relative abundance versus <0.1 in natural turf; p value < 0.001). In natural turfs, *Flavobacterium*, *Janthinobacterium*, *Brevundimonas*, *Agrobacterium* and *Bacillus* (relative abundance: 11.1 %, 7.8 %, 6.4 %, 5.9% and 4.7%, respectively) were also detected.

The analysis of potentially pathogenic genera resulted in a higher relative abundance of OTUs corresponding to opportunistic bacteria

![Fig. 3. a) Principal coordinate analysis (PCoA) scatterplot of the normalized relative abundance of all samples, divided by type of material (red: synthetic and green: natural). Data are plotted at the genus-level classification. b) Partial least square-discriminant analysis (PLS-DA) depicts Pearson distance between different samples using phylogeny distribution based on 16S rRNA genes. Samples are, respectively, coloured according to sampling points.](image)

**Fig. 3.** a) Principal coordinate analysis (PCoA) scatterplot of the normalized relative abundance of all samples, divided by type of material (red: synthetic and green: natural). Data are plotted at the genus-level classification. b) Partial least square-discriminant analysis (PLS-DA) depicts Pearson distance between different samples using phylogeny distribution based on 16S rRNA genes. Samples are, respectively, coloured according to sampling points.

![Fig. 4. Summary of bacterial community abundance of the associated with type of material at each sampling point. To simplify community representation, OTUs less than 1% were discarded and count in the other. While each surface displays a unique community structure, surfaces were similar across all facilities.](image)

**Fig. 4.** Summary of bacterial community abundance of the associated with type of material at each sampling point. To simplify community representation, OTUs less than 1% were discarded and count in the other. While each surface displays a unique community structure, surfaces were similar across all facilities.
more in synthetic (23%) than in natural samples (13%) as summarized in Fig. 6. Indeed, samples from synthetic fields showed OTUs related to human associated bacteria, so that taxa that are commonly found in the human microbiome, were found more frequently, including members of the families Burkholderiaceae, Pseudoxantomonadaceae, Staphylococcaceae and genera Staphylococcus. Moreover, the relative abundance of potentially pathogenic bacteria was independent from the sampling point along the field but significantly varied between the different sport facilities (ANOVA, $F = 2.2$), suggesting a role for the general environment, anthropic contamination, everyday use and management of the playgrounds.

4. Discussion

The chemical and physical risks related to the use of synthetic fields have been investigated for several years, but the biological aspects were not yet fully clarified (CDC, 2003; Kirkland and Adams, 2008). The complexity of the novel materials used for synthetic fields can represent a condition for harboring specific bacteria communities. The role of infill structures was even associated with the accumulation of potential pathogenic organisms and the infectious risk after injuries (Kirkland and Adams, 2008; Cohen, 2008; Bass and Hintze, 2008). 16S amplicon sequencing analysis of synthetic and natural fields confirmed the presence of pathogens but also revealed the existence of a specific microflora (Fig. 5). Interestingly, unknown sequences were over three times more frequent in synthetic vs natural turfs (about 22% vs 7%, respectively), suggesting a specific unknown component present in artificial carpets respect to the well characterize microflora reported in soil and grass (Giampaoli et al., 2014; Nurulita et al., 2016).

This is the first study using 16S amplicon sequencing to characterize the microbiota of soccer fields turfs. The observed data suggested a possible microbial signature own of synthetic vs natural fields. Therefore, athletes, workers and users are exposed to different microbial communities based on the composition of the carpet. The 16S amplicon
sequencing approach allowed also the detection of sequences corresponding to common microbial indicators (e.g. *Staphylococcus*), in agreement with other studies performed in sport facilities using traditional culture-based methods (CDC, 2003; Kirkland and Adams, 2008; Cohen, 2008; Bass and Hintze, 2008). We would have expected synthetic turfs as an adverse environment for microorganisms, but the whole of the observed results showed no different trends in richness and biodiversity distribution of taxa respect to natural grass. The synthetic soccer fields exhibited even a moderately higher mean Shannon’s values. A possible explanation can be found in the contamination of the infill with organic materials, but also in the presence of carbohydrates, amino acids, aliphatic and aromatic acids, fatty acids, that can be released and likely to be a driving force in the structure of the microbial biodiversity within the artificial niche (Nurulita et al., 2016; Prescott and Grayston, 2013; Xue and Huang, 2014; Krashevska et al., 2015). No major differences were observed in alpha-diversity index, but the dissimilarity was evident when using the beta-diversity indicators, supporting a 16S signature approach in characterizing synthetic turfs. Phyllosphere genera were observed in the natural turfs (e.g. *Janthinobacterium*, *Agrobacterium*, *Variovorax*, *Pedobacter*) as expected because of the presence of soil and grass (Simon et al., 2019; Hassani et al., 2018). Several of these bacteria were detected also in synthetic turfs (Figs. 4 and 5), even if less representatively. Being ubiquitous, they could easily contaminate the artificial carpets becoming part of their microbial community, as observed on the surfaces of other synthetic matrices. Interestingly, bacteria from different sources can be found in synthetic turfs, but not conversely in natural ones. Different synthetic materials already were shown to provide a cozy microenvironment to harbour bacteria from anthropic, animal (e.g. *Staphylococcus*, *Streptomyces*, *Nocardioles*, *Hymenobacter*), or other natural sources (Williamsia, *Chryseobacterium*, *Rhodococcus*) (Mafu et al., 1990; Carniello et al., 2018; Sharma et al., 2018; Masoud, 2017). Therefore, a major factor driving beta-diversity variance in artificial surfaces may likely be due to contamination with human sweat or saliva as well as from the natural microflora in the surrounding area. This was not observed in natural turfs probably due to the competition driven by the rich endophytic microflora (Simon et al., 2019; Hassani et al., 2018; Mafu et al., 1990). Mesophilic bacteria, including pathogens, were detected more frequently in the penalty area and centre circle of synthetic turfs, even if the analysis of similarities for the several sampling points showed no changes in microflora profile. These results suggest that microbial communities fluctuate around a common biodiversity centroid, as already reported for other sport plants (Wood et al., 2015). However, within the same facility clear differences can be observed between different sampled areas. The whole of observed results suggests that in synthetic fields the microbial community structure is primarily defined by the anthropic contamination. Management, use, and maintenance of the facility may also play a major role in determining the microbial load and its composition. Infill materials can represent a potential source for bacterial growth posing putatively higher infection risks respect to natural fields, as previously reported for cases of cutaneous infections in soccer players using synthetic turfs (CDC, 2003; Kirkland and Adams, 2008; Cohen, 2008). The microbiota is not an absolute entity, but it represents the result of a complex interaction between the availability of natural microorganisms, the properties of that ecological niche and the influence of different environmental factors. In particular, turfs microbiota can be influenced by local factors (e.g. maintenance products e.g. detergents or pesticides, respectively for synthetic vs natural carpets) or other external factors (e.g. anthropic, animal or environmental pollutants). Some biological pollutants are traceable by microbiota analysis and were detected as possible contaminants in the synthetic turfs (e.g. bacteria of human, animal or soil origin). Otherwise, the microbiota itself can represent a promising approach for detecting traces of contaminants such as biological fluids, feces, plants or other contaminants in different materials and matrices (Valeriani et al., 2018a; Miletto and Lindow, 2015; Leung and Lee, 2016; Mucci et al., 2019). However, exposure to several chemical factors such as volatile organic compounds, particulate matter, polycyclic aromatic hydrocarbons as well as physical factors including ultraviolet radiation, temperature, humidity may represent interfering factors in microbiota formation and stability. A specific issue concerns the micro-conditions within the infill structure of different synthetic materials after exposure to different external factors. Our data focus on the 16S signature of the microbiota and are not so extended to address all the different raising issues. Results from samples coming from different areas, show the presence of a common core structure of the microbiota in
synthetic turfs but cannot provide significant evidence for possible differences due to the complexity of the exposure to external factors. Further and more extended studies are required to address this issue in different playgrounds, starting also from the availability of the present report and available dataset. The 16S amplicon sequencing characterization of turf surfaces may represent a new marker for studying the biological component of synthetic turfs and finally improve management and hygiene in environments for sport and recreational facilities.

5. Conclusions
Soccer playgrounds surfaces are characterized by their own microbiota, showing a different 16S amplicon sequencing signature between natural and artificial turfs. For the first time we report a microbiota analysis of turfs commonly used for playing soccer, football, rugby or other sports as well as recreational and urban playgrounds. Synthetic soccer fields harbor a microbiota from anthropic and environmental sources whereas the traditional natural grass carpets show a soil-related microbial community. Understanding the microbial component in different materials will eventually provide information on their ecology and on the potential health impact of exposed athletes or maintenance workers, both indirectly exposed and through possible injuries. In addition to the several studies addressing the physical and chemical properties related to the synthetic turfs, here we report some data on the biological component and on the application of high throughput sequencing on DNA samples from playground surfaces. Since synthetic fibers made to look like natural grass are often used also for other recreational or furnishing decoration purposes in private and public areas, further advances in the field may provide knowledge for risk assessment and tools for appropriate management and maintenance of synthetic turfs.

Declarations

Author contribution statement
Frederica Valeriani: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Lory Marika Margarucci: Analyzed and interpreted the data.
Gianluca Gianfranceschi, Antonello Ciccarelli, Filippo Tajani: Performed the experiments.
Nicolina Mucci, Maurizio Ripani: Conceived and designed the experiments.
Vincenzo Romano Spica: Conceived and designed the experiments; Wrote the paper.

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The authors declare no conflict of interest.

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