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Cotton-strip assays: Let's move on to eco-friendly biomonitoring!

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ABSTRACT

There is increasing recognition that functional bioindicators are needed for ecosystem health assessments. In this perspective, cotton strip assays are widely considered as a standard method to account for organic matter decomposition in streams. However, cotton cultivation and manufacture raise both environmental and societal dramatic issues that are – in our opinion – irreconcilable with the objectives of bioindication. In this study, we assessed the relevance of four alternative – eco-friendly – textiles (made of organic cotton, hemp and linen) by comparing their chemical composition and degradation rates in six streams. Chemical composition exhibited low variations among textiles, but contrasted sharply with the expectation that cotton is mostly composed of cellulose. Moreover, surprisingly high nutrient (0.49% N) contents occurred in the conventional cotton strips compared with the organic textiles (N < 0.12%). All textiles provided similar degradation rates across the six streams, meaning that they could be interchangeably used as alternatives to conventional cotton strips. We thus call for the adoption of such ethical and eco- friendly tools as 'next-generation' indicators for the functioning of stream ecosystem.

Keywords:

Cotton strip assay
Functional indicators
Streams
Environmental friendly
Hemp
Linen

1. Introduction

The global trend of decreased water quality (Vörösmarty et al., 2010) and aquatic biodiversity worldwide (Dudgeon et al. 2010) urges the development of reliable and standardized indicators of the ecological status for water bodies. Historically based on the species composition of aquatic communities, mounting evidence indicates that bioindication approaches could be enriched by the quantification of ecosystem functions (i.e. functional indicators), as straightforward links to ecosystem services. The decomposition of terrestrial leaf litter – a key process in many streams – has been recommended for that purpose (Gessner & Chauvet, 2002; Chauvet et al., 2016), since it is known to be largely affected by anthropogenic perturbations such as acidification (Ferreira & Guérold, 2017), hydromorphological alterations (Colas et al., 2017), or contamination by nutrients (Ferreira et al., 2015), pesticides (Rasmussen et al., 2012), and heavy metals (Ferreira et al., 2016). The use of litter decomposition as a functional indicator, however, poses some issues of standardization, since variations in litter quality (even within species) has a tremendous influence on decomposition rates (Lecerf & Chauvet, 2008). This shortcoming can be overcome by the use of a standard cellulosic substrate (e.g. cotton) as a surrogate for organic matter decomposition (Tiegs et al., 2007). Cotton-strip assays repeatedly provided satisfactory results as an indicator of stream ecological status (Imberger et al., 2010; Tiegs et al., 2013), and are on track to become a key routine indicator of aquatic ecosystem health (Tiegs et al., 2019).

Cotton textile is produced from shrubs belonging to the genus *Gossypium* (Malvaceae). Its cultivation heavily relies on fertilizers, pesticides and irrigation, which raises both environmental and societal issues (Chen & Burns, 2006; Chapagain et al., 2006). A well-known example is the drying, pollution and salinization of the Aral Sea that followed the exponential development of cotton production during the 1960s' (Aladin & Potts, 1992; Micklin, 2007). Not only the cultivation, but also the processing of fibers to fabrics leads to the abstraction and/or contamination of surface waters (Chapagain et al., 2006). Other consequences of cotton production include the exposure of cotton growers to pesticides, which causes health and fertility issues (e.g. Rupa et al., 1989,1991) for which the only alternative seems to be the use of transgenic cotton (Hossain et al., 2004; Morse et al., 2006), a remedy that is also subject to controversy (Liu et al., 1999; Dhurua & Gujar, 2011). Ironically, cotton production is mostly distributed in areas where water abstraction and pollution (e.g. arid climates), access to health care, and costs associated with chemicals or transgenic seeds supply (e.g. developing countries) are a paramount concern for ecosystems and human populations.

An improvement of the cotton strip assay could thus be achieved by using a more sustainable substrate derived from organic production chains, including organic cotton and/or other textile fibers such as hemp (*Cannabis sativa* L.) and linen (*Linum usitatissimum* L). Hemp and flax cultivation is considered environmental friendly (EEA, 2007; Piotrowski & Carus, 2011) as it requires lower pesticides (particularly hemp), fertilizers (particularly flax) and irrigation than cotton. In this study, we compared the degradation of 'conventional' cotton with various organic textiles (cotton, hemp, linen and a mix of hemp and cotton) in 6 headwater streams. We aimed to determine if they would provide satisfactory alternatives to 'conventional' cotton materials as bio-indicators, i.e comply with the following: exhibit degradation rates that are similar and equally affected by environmental conditions (i.e. exhibiting similar variations across environmental contexts) than 'conventional' cotton. Finally, we expected that differences in the degradation rates among textiles could be related to contrasting chemical composition.

2. Methods

The different textiles included 'conventional' unbleached cotton (CFT E-222, Testfabrics, St Gallen, Switzerland) as well as several organic textiles certified by 'Global Organic Textile Standards' label, including cotton (13/CT-001, La Cantate du Chanvre, Dole, France), hemp (POLLEN, Naturellement Chanvre, Coudeyras, France) linen (Bio Tissus, Plouzané, France) and a cotton-hemp mix (5TX-3000, La Cantate du Chanvre, Dole, France). All exhibited similar grammage (200-250 $g.m^{-2}$). The chemical analysis of textiles was performed on ground material (Retsch MM400 ball mill). Carbon and nitrogen contents were determined using a CHN analyser (Carlo Erba NA 2100), phosphorus was determined spectrophotometrically after digestion in persulfate (Ebina et al., 1983), and fiber content using the Goering & Van Soest method (Goering & Van Soest, 1970). Results are provided in Table 1.

Strip assays were performed following Tiegs *et al.* (2013). Strips of 2 × 8 cm made of each textile were deployed in 6 headwater streams that drain forested catchments and are located in the northern Vosges regional park (Zinsel catchment area, Baerenthal town). Streams were circumneutral (pH = 6.1–7.6) and exhibited contrasting nutrient concentrations representative of the study area (PO_4^{2-} 0.004 – 0.120 mg L^{-1}; NO_3^- 1.15 – 8.89 mg L^{-1}), as assessed year-round ($n = 4$ analyses) prior to the beginning of the experiment. In each stream, strips made of each different textiles were deployed and retrieved after 10 and 20 days (2 sampling dates). With 5 replicates per stream × textile combination, our design involved the deployment of 6 (streams) × 5 (textiles) × 2 (dates) × 5 (replicates) = 300 strips. After retrieval, strips were promptly and gently cleaned into 95% ethanol for 30s, transported to the laboratory and dried in aluminum pans at 40°C for 30 hours. Maximal tensile strength was then determined using a digital force tester (AMETEK CS 225), as well as on 5 control (non-exposed) strips per textile that were cleaned and dried following the same procedure as experimental strips. For each replicate, the calculation of tensile strength loss was based on the 2 sampling dates using a regression between tensile strength (initial, at day 10 and day 20) and time. It was expressed as the percentage of initial (i.e. control) tensile strength loss per day (% day^{-1}). Correlations were used to compare the loss of tensile strength between each textile and the ‘conventional’ cotton, and to analyze the relationships between tensile strength loss and textile chemical composition. Moreover, a ANOVA was used to test for the effects of stream identity, textile and stream × textile interaction on the loss of tensile strength. Compliance with normality and homoscedasticity assumptions was checked graphically. All statistics were performed using R 3.3.1 (R core team, 2016).

3. Results

The average tensile strength loss across streams and textiles was 4.20 % day^{-1} ± 2.0 (*SD*). It varied among textiles ($F_{4,120} = 13.8$, $P < 0.001$), with a significantly higher loss for hemp (TukeyHSD; $P < 0.001$) (Table4 1). Differences also occurred between streams ($F_{5,120} = 25.9$; $P < 0.001$), but no significant stream × textile interaction occurred ($F_{20,120} = 0.9$; $P = 0.535$), indicating that the tensile strength losses of different textiles were similarly affected by stream characteristics. Significant correlations (Fig. 1) indicate a relatively high similarity in the degradation of 'conventional' cotton and alternative textiles, with the highest similarity found for the organic cotton (Fig. 1a), and the weakest correlation for linen textile (Fig. 1c). Chemical composition exhibited limited variation across textiles, though the conventional cotton exhibited the lowest cellulose and the highest nutrient content (Table 1). No significant correlation was found between textile chemical contents and tensile strength losses.

4. Discussion

Our results suggest that all organic textiles we tested could be considered as eco-friendly alternatives to the conventional cotton strips for the monitoring of functional processes in streams. Though slight differences occurred across textiles (higher degradation for hemp), tensile strength loss of our five textiles ranged within the same order of magnitude as previously reported for 'conventional' cotton strips (e.g. Tiegs et al 2007, 2013; Imberger et al., 2010; Clapcott et al., 2010). Most importantly, patterns of tensile strength loss were similar across streams and textiles (i.e. no significant interaction between stream and textile identity), which means that all textiles were affected similarly by the environmental conditions covered in our experiment. This gives a first hint that they could be similarly appropriate for biomonitoring purposes. The least satisfactory fabric, which should probably be excluded as a potential bioindicator, was the linen textile. Its tensile strength measurement was less repeatable than for other textiles, leading to high variability in the initial tensile strength (Table 1) and a comparatively weak correlation with the 'conventional' cotton tensile strength loss (Fig. 1).

All substrates exhibited very similar contents of lignin (almost nil) and cellulose (Table 1). The 'conventional' cotton, though, exhibited a surprisingly high nutrient content compared with other textiles. The root cause of this difference is unknown. Since conventional cotton is the only non-organic fabric, we strongly suspect that they are due to chemicals or processes that are banned in eco-friendly textile production (see GOTS Standard Committee, 2017). Higher nutrient content in cotton, for instance, can originate from fertilizers and chemicals used for cotton cultivation (Boquet &

Breitenbeck, 2000; Ferrigno et al., 2017), but also from fiber processing and textile finishing (Babu et al., 2007). Lower contents of cellulose than expected in all substrates – and particularly in 'conventional' cotton – are more intriguing, and contrast sharply with the cellulose content of the raw cotton fiber (88–96% cellulose; McCall & Jurgens, 1951). Surprisingly, we could not find any previous analysis of cotton chemical composition despite the widespread use of the method in both terrestrial and aquatic ecology (Harrison et al., 1988; Tiegs et al., 2013).

With this paper we are calling for the use of ethical and eco- friendly tools as 'next-generation' functional indicators. In the case of cotton strip assays as indicators of stream ecosystem functioning, our results show that organic cotton could be used as an eco-friendly alternative to conventional cotton fabric. Clearly, amounts of cotton used for bioindication purposes are a negligible fraction of the total cotton world production. However, using ecological indicators aims to assess – and improve – the ecological status of ecosystems. This goal cannot be meaningfully achieved if these tools cause any societal or environmental damage – no matter how small, as far as satisfactory alternatives are available. We share the idea that weighing the benefits against the environmental and societal costs of the tools and methods we use is probably one of our greatest responsibility as scientific ecologists (e.g. Rosen, 2017).

5. Conclusions

- Organic textiles decompose at similar rates than conventional cotton across 6 different streams.
- The relatively low cellulose and high nutrient content exhibited by the conventional cotton fabric bring into question its use as a 'pure cellulose' standard substrate.
- Organic textiles, and in particular organic cotton, can be used as an eco-friendly alternatives to conventional cotton for the cotton-strip assay in streams.

Competing financial interests

The authors declare no competing financial interests

Author contributions

JJ, FC and FG designed the study, JJ and FG carried out the experiment and data analysis. JJ, FC and FG wrote the manuscript.

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Fig. 1. Correlations between the average (± *SE*) tensile strength loss of 'conventional' cotton strips and the tensile strength loss of each of 4 alternative textiles in the 6 streams. The regression lines (solid lines) as well as Pearson correlation coefficients and associated p-values are provided. Numbers are the ranks of the streams according to the average rates of tensile strength loss for conventional cotton strips.

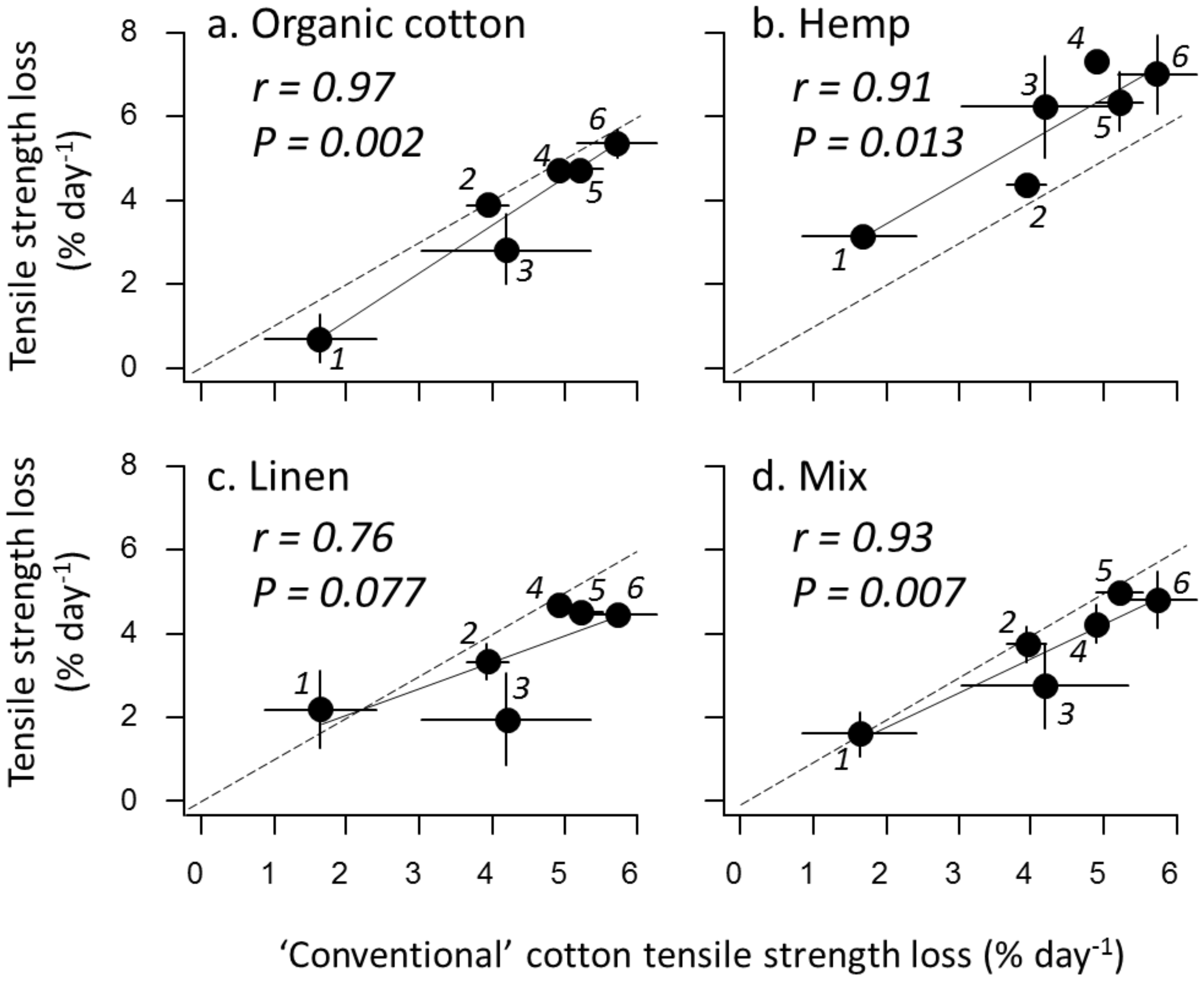
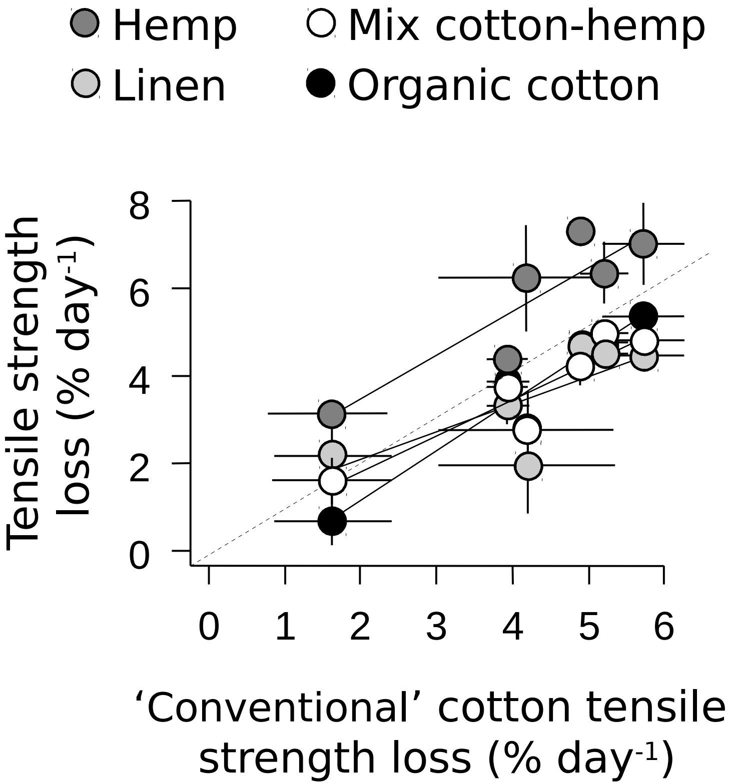


Table 1

Properties of the 5 different textiles used in the experiment (average ± *SD*). Results are expressed in % of dry mass except tensile strength loss (% day $^{-1}$) and initial maximal tensile strength (N).

	Carbon $n = 5$	**Nitrogen** $n = 5$	**Phosphorus** $n = 2$	**Proximate cellulose** $n = 5$	**Proximate lignin** $n = 5$	**Tensile strength loss** $n = 30$	**Initial max tensile strength** $n = 5$
Cotton	41.92 ± 0.04	0.486 ± 0.013	0.032	61.92 ± 7.29	0.00 ± 0.00	4.27 ± 1.87	234.3 ± 8.1
Organic cotton	42.45 ± 0.04	0.094 ± 0.009	0.007	74.68 ± 6.80	0.11 ± 0.08	3.70 ± 1.83	233.9 ± 17.2
Hemp	42.49 ± 0.13	0.098 ± 0.008	0.015	74.50 ± 5.49	0.50 ± 0.03	5.75 ± 2.06	247.9 ± 23.2
Linen	42.59 ± 0.06	0.120 ± 0.010	0.012	70.91 ± 6.05	0.53 ± 0.16	3.53 ± 1.70	270.5 ± 89.9
Mix	42.28 ± 0.09	0.066 ± 0.006	0.011	63.75 ± 4.58	0.23 ± 0.29	3.69 ± 1.71	191.5 ± 10.0



Hemp
Mix cotton-hemp
Linen
Organic cotton
Tensile strength loss (% day^{-1})
8
6
4
2
0
0
1
2
3
4
5
6
'Conventional' cotton tensile strength loss (% day^{-1})