

Vaginal bacterial communities in relation to the physiological status of genital tract of healthy reproductive-age women

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INTRODUCTION

The vaginal environment of healthy reproductive age women is considered to be acidic with mean pH measures of 4.2 - 4.7 (e.g. Ravel *et al.* 2010; Rönqvist *et al.* 2006). Acidic environment is known to be predominantly maintained and regulated by certain vaginal bacteria, especially lactobacilli that produce H₂O₂, bacteriocins and bacteriocin-like substances (Boskey *et al.* 1999, Rönqvist *et al.* 2006). Higher pH values have been associated with increased risk of vaginal disorders such as bacterial vaginosis (Mania-Pramanik *et al.* 2008).

All the previous knowledge has been gathered using classical microbiology methods that allow us to study cultivable bacteria, but do not say much about the whole microbial picture. A few recent studies using novel second generation DNA sequencing methods (e.g. Ravel *et al.* 2010) have shown the relationship also between vaginal pH values and different bacterial community composition.

The aim of current study was to investigate whether vaginal pH values are related to different vaginal microbial community structure parameters in healthy reproductive age Estonian women using HTP pyrosequencing. As well as to analyze the variance of bacterial taxa at the different pH ranges.

RESULTS

Vaginal pH varied in the range of 3 to 9 among 451 studied women with the mean value of 4.63 (± 0.76) (Figure 1). Shannon diversity index of vaginal bacterial communities correlated positively with pH measures ($t=3.64$; $p=0.0003$) as shown by linear regression model, where age was included as factor (Figure 2). Taxonomic richness was not influenced by different vaginal pH measures ($t=0.41$; $p=0.68$) (Figure 3). Figure 4 shows the effect of vaginal pH on the levels of the eight most abundant operational taxonomic units (OTUs) detected in the samples. Relative abundance of lactobacilli decreased with the increase in vaginal pH measures (Table 1). On the other hand, significant positive correlation of vaginal pH measures and relative abundance of genera *Gardnerella*, *Prevotella*, *Atopobium* and *Streptococcus* was detected. The amount of bacteria from genus *Sneathia* had positive tendency to increase with vaginal pH measures and relative abundance of genera *Ureaplasma* and *Bifidobacterium* was not significantly affected by vaginal pH (Table 1).

Table 1. Linear regression for estimating the relationships between the relative abundance of the particular bacterial taxa and vaginal pH measures.

OTU	t-value	p-value
<i>Lactobacillus</i>	-8.49	3.38e-16
<i>Gardnerella</i>	6.11	2.18e-09
<i>Prevotella</i>	4.0	2.12e-05
<i>Atopobium</i>	2.68	0.0076
<i>Streptococcus</i>	4.22	2.7e-05
<i>Sneathia</i>	2.9	0.037
<i>Ureaplasma</i>	1.87	0.062
<i>Bifidobacterium</i>	1.6	0.11

MATERIAL AND METHODS

All the 451 human subjects in the study were healthy pre-menopausal non-pregnant ethnically homogenous women 15-44 (mean 31.1 ± 6.4) years of age without complaints and symptoms in urogenital tract. The lack of infections was confirmed by gynecologists during the physical examination of patients while samples were taken from individual's vaginal fornix. Vaginal pH was measured during sampling with Merck pH-indicator strips 2,0-9,0 (0,5 unit increments). Sampling period was June - October 2010. The individual samples were stored at -20° C until extracting total DNA with BioSprint 96 DNA Blood Kit (Qiagen). Pyrosequencing analysis of the 16S rRNA gene V2 hypervariable region for parallel tagged sequencing on the 454@ Platform were performed by Roche/454 Life Sciences sequencer (GS junior System). Primers used for amplification were 8F 5' TTGGCAGTCT-CAGnnnnnnnnAGTTTGATCCTGGCTCAG 3' and 357R 5' GTCTCCGACTCAGnnnnnnnnCTGCT-GCCTYCCGTA 3' (McKenna *et al.* 2008). The 8-bp barcode sequence is marked as „n“s.

The sequences in the initial pretrimmed dataset (757282 sequences) were checked for chimeric artifacts, pyrosequencing noise and non-unique sequences were removed. The reference sequences of aligned 16S rDNA were obtained from SILVA ribosomal RNA database. Taxonomic assignments were done by Ribosomal Database Project (RDP) Naïve Bayesian classifier. OTUs with a 97% identity threshold were defined using the average neighbor clustering algorithm of mothur 1.19.1 software (Schloss *et al.* 2009). Statistical analyses were performed with the R 2.13.1.

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CONCLUSIONS

This is the first study showing correlations between bacterial community composition and pH in the population of asymptomatic women in Estonia.

Vaginal pH measures had significant effect on the diversity of bacterial community.

Relative abundance of lactobacilli decreased with the increase in vaginal pH, which is in concordance with previous studies.

Results showed positive correlation between vaginal pH measures and relative abundances of four genera previously associated with bacterial vaginosis.

Relative abundance of genus *Streptococcus* had positive tendency to increase with higher pH.

There arises question, if the patients with extreme pH values had asymptomatic vaginal infections. However, vaginal bacterial communities need to be analyzed further also in species level to bring clarity into this question.

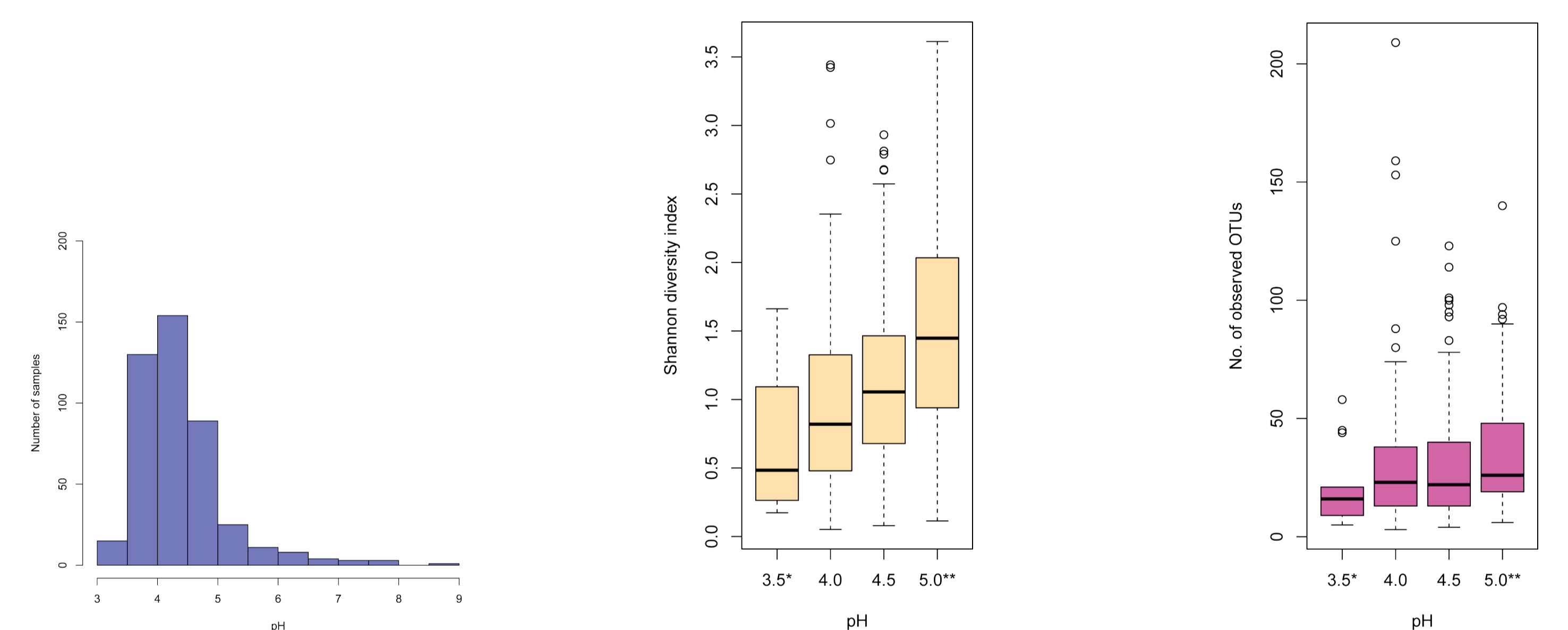


Figure 1. Distribution of vaginal pH measures among studied women.

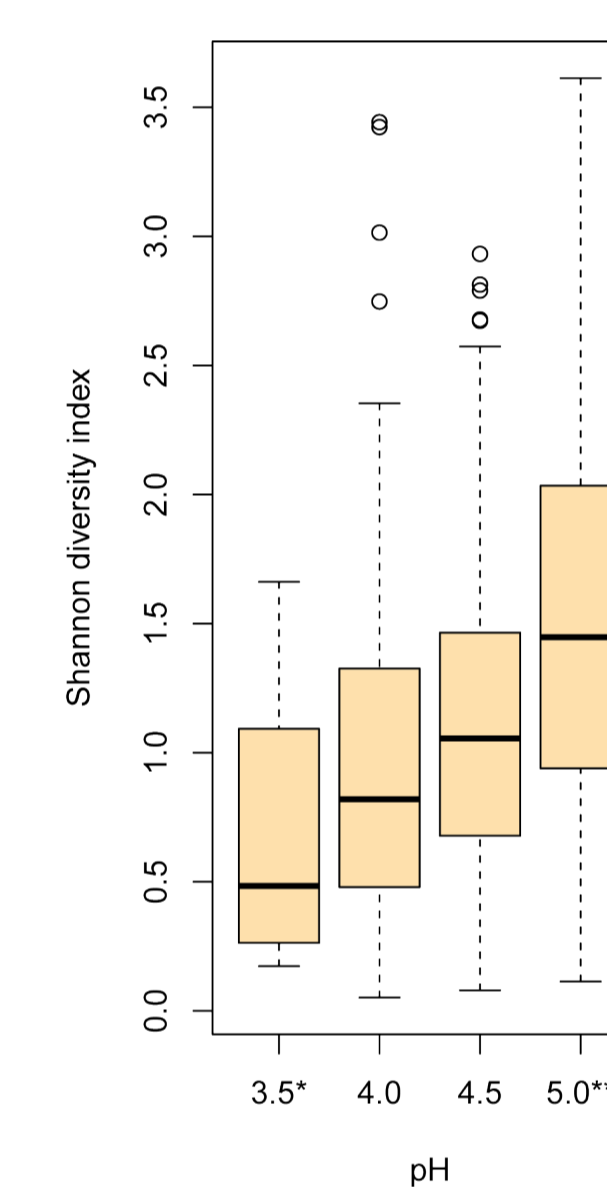


Figure 2. The correlation between vaginal pH measures and Shannon diversity index. *indicates to pH values 3.0 and below ** indicates to pH values 5.0 and above

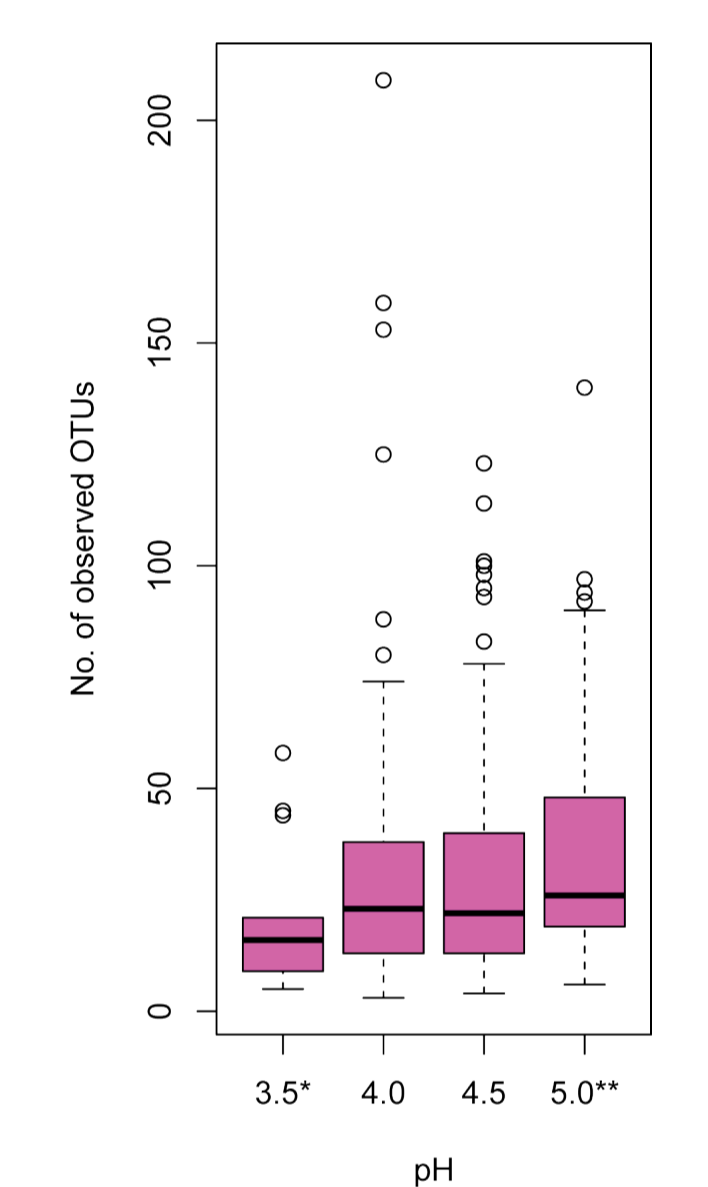


Figure 3. The correlation between vaginal pH measures and number of observed OTUs. *indicates to pH values 3.0 and below, ** indicates to pH values 5.0 and above

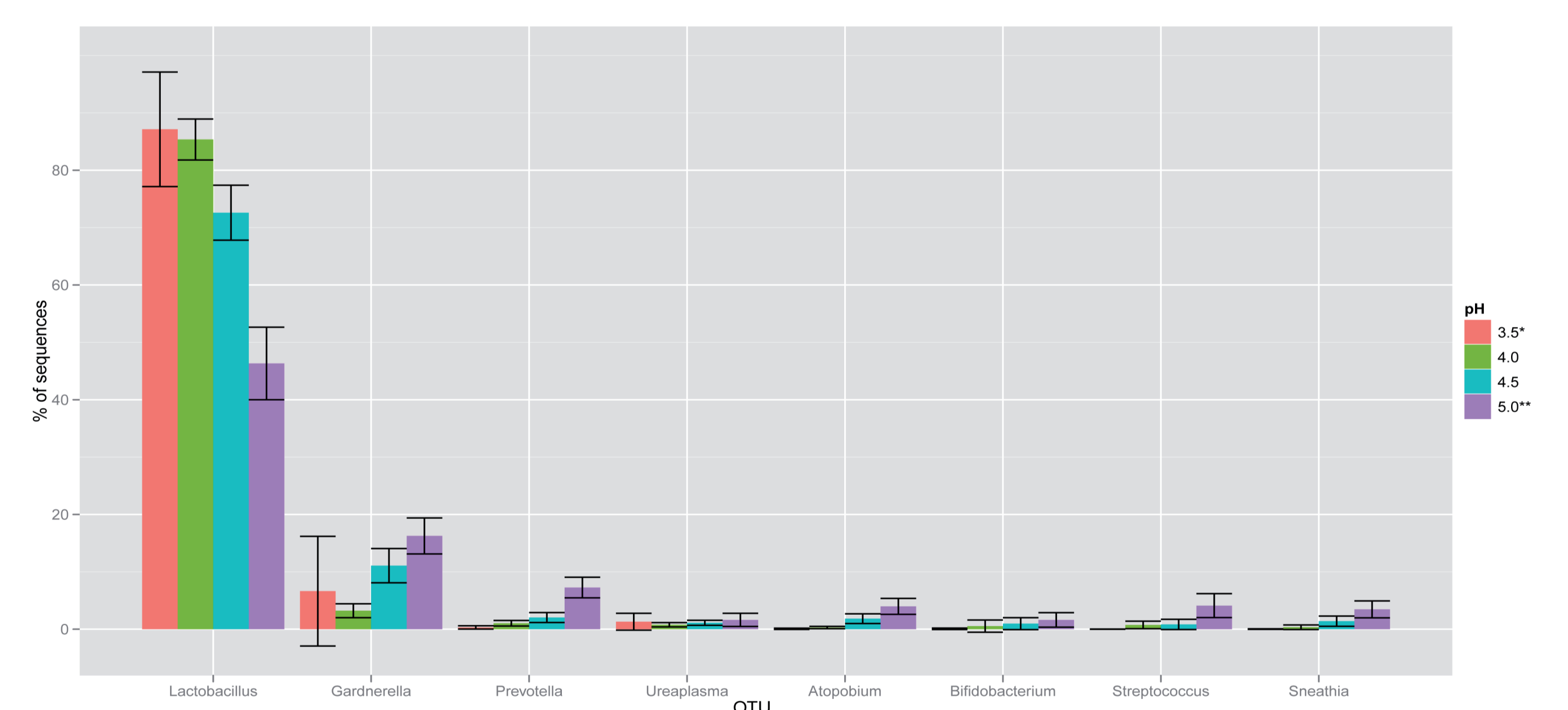


Figure 4. Relative abundance (mean and 95% confidence interval) of the bacterial taxa in different vaginal pH measures shown as percentage of sequences. *indicates to pH values 3.0 and below ** indicates to pH values 5.0 and above

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